BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

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Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

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Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
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Key: APC: Aerobic Mesophilic Counts
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Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
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Makwalla	68	13.60 ± 8.7062
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Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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MATERIALS AND METHODS

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

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A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
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Key: APC: Aerobic Mesophilic Counts
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Sampling Area	TCC MPN/g	Mean ± STD
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Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
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Rinji	3	60
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Key: APC: Aerobic Mesophilic Counts
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Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

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However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

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Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

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Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

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Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
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Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
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Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

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Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

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Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

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Rinji	3	60
Tosaro	2	40
Tsohuwar Kasuwa	1	20
Total	16	

CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
2. Monitoring of microbiological contamination of *Tsire* during preparations, packaging and marketing is essential to ensure product of good microbiological quality for consumers' health.
3. Government should establish regulatory bodies responsible for inculcating hygiene habits to the local producers and vendors in order to prevent instant, cross and post processing contaminations by microbial pathogens.
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BAJOPAS Volume 13 Number 1, June, 2020 **http://dx.doi.org/10.4314/bajopas.v13i1.7**

Bayero Journal of Pure and Applied Sciences, 13(1): 40 - 45
Received: September, 2019
Accepted: April, 2020
ISSN 2006 – 6996

BACTERIOLOGICAL QUALITY OF STICK MEAT (*Tsire*) SOLD IN GARKO LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT
Stick meat, locally called Tsire in Hausa Language, is a significant portion of the diet of a large active population of Northern Nigeria. However, because of the handling and processing methods by the manufacturers as well as the hawking system of stick meat, the meat product may be exposed to both pre- and post-processed product contamination, and thus may poses danger to public health. Therefore, considering the role of Tsire in improving nutrition and increase household income to the populace, the need to improve the processing, distribution and overall quality of the product is simply indispensable. A total of 30 samples (5 samples from each of the 6 sampling points) of Tsire were collected randomly in Garko Town. Aerobic plate counts (APCs) and Total coliform counts (TCCs) of Tsire samples were determined using standard microbiological methods. APCs ranged from 1.02 × 10^5 to 4.00 x 10^5 CFU/g; while TCCs ranged from 8MPN/g to 68 MPN/g. From the 30 samples screened, 16(53.33%) bacteria namely, Campylobacter 2(6.66%), Escherichia coli 6(20%), Klebsiella pneumoniae 2(6.66%), Salmonella spp. 1(3.33%), Shigella 3(10%) and Staphylococcus aureus 2(6.66%) were detected. E. coli has the highest isolation rate of 6(20%), while Salmonella spp. had the least frequency of 1(3.33%). The distribution of isolated organisms within the study area were as follows: Dausai, 4(80%), Kofar kudu, 4(80%), Makwalla, 2(40%), Rinji, 3(60%), Tosaro, 2(40%), and Tsohuwar Kasuwa, 1(20%). The results have indicated that the Tsire was contaminated with bacteria thus exposing the product to fast deterioration, spoilage and also a vehicle for food borne illness, suggesting for adequate hygienic practices at all the stages, prior to consumption, to ensure safety of the meat product.
Key words: APCs, TCCs, Contamination, Microorganisms, Garko

INTRODUCTION

Nigeria is one of the developing nations with inadequate food supply and most often deficient in protein content (FAO, 2019). Nigerian's average animal protein intake per head per day is estimated at 7.9g as against 35g recommended value by Food and Agricultural Organization (Mubarak *et al.,* 2016). These low levels of animal protein intake create great concern as it affects the nutritional status of Nigerians. Hence, there is the need for sufficient supply of animal protein from energy rich animal products to satisfy the nutritional requirements for Nigerians.

Meat is one major source of animal protein largely composed of water, protein and fats which can make it susceptible to microbial contamination within short time leading to spoilage (Apata *et al*., 2013; Mgbemere *et al.,* 2011). Meat is mostly eaten after it has been cooked or processed in a variety of ways such as sun drying; smoking and roasting with or without fortifications (Borch *et al.,* 1996). In Nigeria meat products such as *Tsire*, *Kilishi* and *Balangu* are locally produced using one or more of these methods in order to meet the nutritional requirements of the teeming Nigerians (Egbebi, 2011).

Tsire is a popular Nigerian traditional processed ready-to-eat roasted stick meat product (Shamsuddeen, 2015). It is sold in public places, along roads, in hotels, parks, quarters and even offices (Falegan *et al.,* 2017). It is prepared from boneless healthy animal flesh such as beef and mutton, spiced with peanut cake, salt, vegetable oil and other flavorings followed by roasting (Shamsuddeen, 2015). Consumption of these products has extended to other parts of African countries such as Ghana, Somali, Cameroun and Chad (Ahmadu and Ibrahim, 2013).

However, evidence of quality control sticking to procedural hygiene by the handlers during preparation and retailing of these products is poorly documented. *Tsire* is of great safety risk because of the fact that there are erratic cases of gastroenteritis and symptoms of food infection after consumption (WHO, 2015). Food borne illnesses are one of the major health problems in developing and developed countries (Razavilar, 2010). According to the World Health Organization, WHO (2015), 0.07% death of the 600 million global burdens of Foodborne illnesses have been reported to be caused by bacteria, viruses, fungi and chemicals. In America 0.27% of the estimated 48 million affected people is hospitalized and about 0.0063% deaths are recorded each year (Scallan *et al.,* 2011). More than 91 million people in Africa fall ill and 0.15%dies each year, making Africa the highest burden bearer of Foodborne diseases per population (WHO, 2015).

Application of a Hazard Analysis Critical Control Point (HACCP) at all stages of meat products preparation is essential in order to ensure its safety. Thus, according to the guidelines of good manufacturing practice, the level of total aerobic bacterial contamination of thermally processed meat products should not exceed 10^4 (cfu g^{-1}). Enterobacteriaceae and faecal coliform contamination in meat products should be within the range of 10^2 - 10^4 and 10 - 10^3 cfu g^{-1}, respectively (Shamsuddeen, 2015). The aim of the study is therefore, to assess the total viable bacterial counts of *tsire* marketed locally within Garko town, and to isolate and identify the organisms at the point of consumption with the intention of promoting public health and food hygiene habits in the Nigerian populace.

MATERIALS AND METHODS

Study Area and Population

Garko is one of the existing 44 local government areas located in Kano south west zone of Kano state with its capital administrative headquarters in the Garko town. It has coordinates 11°39′N 8°54′E, and an area of 450 km². The projected population of Garko local Government was 225,300 according to the National Population Census report (2018). Garko is known for agricultural activities both farming and animal husbandry. It is popularly known for large scale rice production. Other food crops include sweet potatoes, cassava, onions, sorghum, millet and sugarcane. Cows, sheep and goats are major groups of animals reared by majority of the populace. Meat products processed, retailed and consumed in Garko include roasted meat (*Balangu)*, dried roasted meat (*Kilishi),* stick meat (*Tsire),* hide and skin pepper soup (*Ragadada*) and minced fried meat (*Danbun-nama).*

Samples Collection

Five samples of *Tsire* products were collected from 6 different identified locations within Garko town in a sterile foil paper and immediately analyzed for the presence of bacteria.

Sample Preparation

Sample preparation for the bacteriological analysis was carried out in accordance with the method described by Atlas (1997). Twenty five grams (25g) of the sample was homogenized in 225ml peptone water using Kenwood blender machine to obtain a 10^1 homogenate. The homogenate was thoroughly shaken and 1ml pipetted into test tubes containing 9ml of peptone water (10^2). The test tubes were further serially diluted to 10^5.

Total Aerobic Mesophilic Bacterial Count

Total Aerobic mesophilic bacterial count was determined using the method described by Abdullahi *et al.* (2004) where 1ml of inoculums from 10^1 to 10^5 dilutions were transferred into duplicate Petri dishes and labeled accordingly. This was followed by pouring aseptically about 15ml of molten nutrient agar. The culture was homogenized by gentle spinning of the plates and allowed to solidify. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were counted. The number of colony forming units per gram of a sample (cfu/g) was obtained by multiplying the average colony number with the inverse of the dilution factor.

Enumeration and Detection of Coliform bacteria

Detection and enumeration of coliform was carried out according to method described by Atlas, (1977). A set of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tubes were autoclaved to expel air and to sterilize. Similarly, 1ml from the diluents 10^1 was transferred to the first 3 test tubes, followed by 1ml from the diluents 10^2 to the second set of 3 test tubes and finally the third diluents 10^3 to the 3rd set of 3 test tubes. All the 9 test tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliform. Negative tubes were further re-incubated for 24 hours. Positive tubes were recorded. Estimate of most probable number of Coliform per gram of sample (MPN/g) was determined by comparing the number of gas positive tubes with the most probable number table.

Identification of Coliform

A loop full of inoculum from gas positive tubes was streaked on to Eosine methylene blue (EMB) agar plate and incubated at 37ºC for 24 hrs. Colonies which formed bluish black color with green metallic sheen, and reddish colonies were isolated on agar slants. Those colonies showing metallic sheen on EMB were sub cultured into tubes of lactose broth and incubated at 45ºC. The tubes were observed after 24hrs for gas production. This is the completed test for fecal coliform. Gram stain and other biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate Utilization tests (IMVIC), Coagulase and Catalase tests were carried out for the Identification and confirmation of isolates.

Procedure for Indole Test

Indole test was carried out by preparing a Tryptone broth drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and incubated at 37° C for 24 hours. Three drops of xylene was added in tubes, shaken vigorously and kept for the separation of two layers. One millilitre of Kovac's reagent was added and the formation of pink colour ring indicates positive Indole test.

Procedure for Methyl Red Test

Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized, inoculated with test culture and incubated at 37°C for 24 hours. Five drops of methyl-red indicator was added to the medium for the formation of red colour.

Procedure for Voges-Proskauer Test

Voges-Proskauer test was carried out by inoculating tubes with the bacterial culture followed by incubation for 48 hours at 37°C. Separate pipettes were used to pipette 1 ml from each culture tube into clean separate tubes. Eighteen drops (0.5 ml) of Barrit's solution A (a-naphthol) was added to each tube containing glucose phosphate broth followed by the addition of an equal amount of solution B into the same tube. The tubes were shaken at 30 seconds interval. A positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking.

Procedure for Citrate Utilization Test

Citrate Utilization Test was carried out by distributing melted agar (Simmon Citrate Agar) in to test tubes followed by sterilization at 121.5°C for 15 minutes. The test tubes were afterward held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Positive test was indicated by color change of the media from green to blue.

RESULTS AND DISCUSSIONS

The results of this study are presented in tables 1, 2, 3 and 4. Among the sampling areas Kofar Kudu had the highest aerobic mesophilic bacteria counts 4.0 x 10^5 cfu/g followed by Rinji 2.72 x 10^5cfu/g, while Tosaro had the least Aerobic mesophilic bacteria counts of 1.02x10^5 cfu/g as indicated in Table 1.Makwalla had the highest coliform count 68 MPN/g followed by Rinji 37 MPN/g. Tsohuwar Kasuwa had the least coliform counts 8 MPN/g, (Table 2). Result from table 1 shows variation in the microbial contents among the samples which suggest variation in their sources, poor handling procedures and contamination from the processing environment. High coliform count and their differences within sampling areas is an indication of poor microbiological quality of the product. Ndahi *et al.* (2013) reported microbial load to be a function of the handling personnel and the environment. The results indicated that the samples were contaminated with bacteria as the counts exceeded the minimum safety level (10^4cfu/g) for members of the Enterobacteriaceae family. However, it might be a reflection of poor hygienic practices which may contribute to health hazard to the potential consumers. Similarly, Inusa and Sa'id (2017); Osimani *et al.* (2015) reported that, the initial microbial content of the raw material play significant role in influencing the final microbial load of the finished product. Isolation of members of the Enterobacteriaceae such as *E. coli, Klebsiella pneumoniae, Salmonella* and *Shigella* signifies danger to the public health. These organisms are capable of producing endotoxins which trigger high fevers in infected individuals, and the enterotoxins which colonize the small intestines and lead to extreme dehydration as a result of vomiting and diarrhea sometimes with severe and fatal outcomes (Amaeze *et al.,* 2016). From the 16 isolated organisms *E. coli* had the highest isolation rate 6(37.5%)(Table 3), this suggest faecal contamination of the products. *E. coli* is a common flora in the gastrointestinal tract responsible for diarrhea and extra-intestinal infections (CDC, 2011). The result is in conformity with the findings of Ndahi *et al.* (2013) and Shamsuddeen (2009). Also presence of *campylobacter* might be due to cross contamination from raw meat to finished product (Tsire). *Staphylococcus aureus* is a normal flora of the skin, nasal, genital, mouth or anal area in both humans and animals without any symptom of an infection (Matthew *et al.,* 2013). Isolation of *S. aureus* in these study concords with the findings of Lucretia *et al.* (2018) from *Suya* sold in Rivers State.

It is also in agreement with the findings of Yousafzai *et al.* (2018); Onuorah *et al.* (2015) and Tijjani and Jumare (2014) from tsire samples in different countries. Presence of *S. aureus* in foods is most of the time an indication of poor human handling, poor environment, unhygienic utensils and equipments used during processing (Igene *et al.,* 2016; Okonko *et al.,* 2013). Infections caused by *S. aureus* are difficult to treat leading to long hospitalization with consequent economic loss (Udobi *et al.,* 2013). These include soft tissue infections, pneumonia, hospital–acquired postoperative wound infections, *Staphylococcal* food poisoning, impetigo and cellulitis (Charlene *et al.,* 2013). Samples obtained from Dausai and Kofar Kudu had the highest isolation rate 4(80%) (Table 4) probably because of the much commercial activities taking place in the area which can lead to environmental contamination.

Table 1: Total Aerobic Mesophilic Bacterial Count of *Tsire* sold in Garko Town

Sampling Area	APC cfu/g	Mean ± STD
Dausai	2.20×10^5	110000 ± 14142.14
Kofar Kudu	4.00×10^5	133333.3 ± 23094.01
Makwalla	1.30×10^5	32500± 7187.953
Rinji	2.72×10^5	90666.67 ± 94769.90
Tosaro	1.02×10^5	51000 ± 15556.35
Tsohuwar Kasuwa	1.75×10^5	35000 ± 11357.82

Key: APC: Aerobic Mesophilic Counts
STD: Standard Deviation

Table 2: Total Coliform Count of *Tsire* sold in Garko Town

Sampling Area	TCC MPN/g	Mean ± STD
Dausai	20	6.67 ± 2.5166
Kofar Kudu	32	16.00 ± 5.6569
Makwalla	68	13.60 ± 8.7062
Rinji	37	9.25 ± 4.7871
Tosaro	10	5.00 ± 2.8284
Tsohuwar Kasuwa	8	4.00 ± 0.0000

Table 3: Organisms Isolated from *Tsire* Sold in Garko

Isolated Organism	Frequency	Percentage (%)
Campylobacter	2	06.66
Escherichia coli	6	20.00
Klebsiella pneumoniae	2	06.66
Salmonella species	1	03.33
Shigella	3	10.00
Staphylococcus aureus	2	06.66
Total	16	

Table 4: Distribution of Organisms within Sampling Area

Sampling Area	No. Isolated	Percentage (%)
Dausai	4	80
Kofar Kudu	4	80
Makwalla	2	40
Rinji	3	60
Tosaro	2	40
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CONCLUSION

Results indicated 4.00 x 10^5 CFU/g as the highest APCs count; while 68 MPN/g was the highest TCCs from the 30 samples analyzed. Among the isolates, *E. coli* has the highest isolation rate of 6(20%). Dausai and Kofar kudu were having the highest bacteria count of 4(80%), each, indicating higher contamination in those locations.

RECOMMENDATIONS

The following recommendations are tenable:

1. Hygienic practices at all stages of production should be improved to safe guard the consumers against potential health hazard.
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4. Awareness and sensitization of local food producers about good hygienic practices in food handling and processing.

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