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Bacteriological Quality Assessment of *Kilishi* Produced in Kunchi Local Government Area, Kano State, Nigeria

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Abstract
Dried roasted sliced meat (*Kilishi*) is a popular meat product in Northern Nigeria that is relished locally and internationally. However, the abundance of a number of nutrients that favour the establishment, growth and proliferations of microorganisms, makes it a vehicle for transmitting food borne illness. This study was aimed at determining the microbiological quality of *Kilishi* in Kunchi Local Government Area, being one of the well-known *Kilishi* production town in Kano State. The experimental lay-out for the study was a completely randomised design in which a total of 15 freshly prepared Kilishi samples (100g each) were collected from three well known retail locations, identified as A (Malikawa Garu), B (Shuwaki) and C (Kunchi town), respectively. The samples were assessed for their microbiological quality according to standard procedures. Results for total aerobic mesophilic bacterial count (APC) cfu/g show Location A had 3.92×10^5cfu/g, Location B had 4.83×10^5cfu/g, Location C 5.43×10^5cfu/g. The results for the total coliform counts(TCCs) revealed30 MPN/g for Location A, 37 MPN/g for Location B and 50 MPN/g for Location C. Biochemical analysis confirmed the presence of *Escherichia coli*, *Klebsiella* specie, and *Staphylococcus* specie in all the three locations, while *Enterobacter* was detected in Location C. Our finding indicates serious contamination of *kilishi* products at retail outlets which could be of public health concern. Therefore, good production practices, packaging and storage were recommended.
Key words: *Kilishi*, APCs, TCCs, Detection, Microorganisms, Kunchi

INTRODUCTION

Meat and meat products make valuable contributions to diets of developing countries due its excellent source of high quality protein, large amounts of minerals, essential vitamins, fats and carbohydrates (Mgbemere *et al.*, 2011). However, in spite of its constant demand, meat is a highly perishable food item due to abundance of a number of nutrients that favour the establishment, growth and proliferations of microorganisms (Wu *et al.*, 2017; Elisabeth *et al,*. 1996). Fonkem *et al.* (2010) noted that, the presence of some of these microorganisms may render it poisonous and unfit for human consumption. This may be the reason why man, has over the decades developed a number of meat preservation techniques that can maintain its stability and increase its shelf-life while at the same time possessing adequate nutritive value and desirable flavour. According to Apata *et al.* (2013) meat post-harvest processing in which properties of fresh meat are modified by the use of one or more seasoning, heat treatment or drying, is one of the ways of preserving it against microorganisms. For instance, dried sliced beef (*Kilishi*), a traditional dried meat product made from meat infused with spices and defatted groundnut paste (Muhammad and Muhammad, 2007; Abubakar *et al.*, 2011; Olusola *et al.*, 2012), is a popular meat product in Northern Nigeria.

Kilishi is prepared by partially drying thin sheets of quality beef in the sun followed by addition of some ingredients before a second period of sun drying and partial roasting (Igene *et al.*, 1990; Musonge and Njolai, 1994). However, it has been shown that the quality of *Kilishi* produced by the traditional processors varies from one producer to the other and from one batch to another by the same producer (Olusola, 2006). Okonko *et al.* (2013) lamented that high ambient temperature, low humidity, shortage of portable water and poor handling practices expose meat products to microbial contamination and rapid deterioration.

According to the Centre for Diseases Control, CDC (2013), consumption of foods contaminated with pathogenic microorganisms or their toxins remains one of the major causes of disease hospitalization, and economic loss in spite of the increasing attention of the

public authorities and consequently of the food operators towards food hygiene and food safety.

This might be the reason why, *Kilishi* industry occupies a delicate position with important public health implications since this sector involves complex procedures which aim at preparing a large quantity of meat product relished locally and internationally.

Among the numerous types of meals served at catering, meat products deserve particular attention because, if not properly processed and preserved, they can be the source of pathogenic microorganisms (Petruzzelli *et al.*, 2010; 2014). This is because effectiveness of the cooking process depends on several factors, including the microbiological quality of raw meats, amount of meat to be cooked, size of the piece(s), cooking method, type of equipment used for cooking and presence or absence of additional ingredients (Fiona, 2017). In addition, efficient protection from different kinds of post-cooking contamination, rapid cooling down and controlled cold storage are all of primary importance for guaranteeing safety of cooked meat preparations (Daelman *et al.*, 2013; Gibbons *et al.*, 2006; Juneja *et al.*, 2001; Mataragas *et al.*, 2008). It is on the basis of the above assertions that, the present study uses congruous number of microbiological analyses to determine the bacteriological quality of dried sliced beef (*Kilishi*) in Kunchi Local Government Area, Kano State, being one of the major *Kilishi* Industry in the State. This is important considering the fact that, *Kilishi* serves as ready to-eat snacks in Kano State and therefore, could be a source of infection to the populace.

MATERIALS AND METHODS

Study Area

The study was conducted at Malikawa Garu, Shuwaki and Kunchi town, all of Kunchi Local Government Area, Kano State with headquarters in the town of Kunchi. It has an area of 671 km² and a population of 111,018 at the 2006 census (National Population Commission, 2006). Kunchi is located on Latitude 12°30′05″N and Longitude 8°16′18″E. Its climate is composed of dry and wet seasons. The main tribes are Hausa and Fulani that are mostly engaged in trading, handcrafts, farming, civil service as well as *Kilishi* production, processing, whole selling, retailing and hawking.

Samples Collection

Freshly prepared dried roasted sliced meat samples (100g each) were collected from three well known selling points in Kunchi Local government area of Kano State. However, the health status of the animals used for preparing the *Kilishi* was not established. Five samples were taken from each of the sampling points. The samples were aseptically placed in a clean, sterile aluminium foil immediately after collection and subsequently transported to the Post Graduate laboratory of the Department of Microbiology, Bayero University Kano. Sampling was conducted according to availability and hygienic practices of processors and the environment.

Sample Preparation and Serial Dilution

The sample preparation was carried out according to FAO (1979) where 25g of sample was weighed and homogenized by blending in 225ml peptone water at 15, 000 – 20, 000 rpm. This was labelled as 1:10 dilution which was also further serially diluted to $1:10^7$.

Total Aerobic Mesophilic Bacterial Count

The total aerobic mesophilic bacterial count was carried out according to Abdullahi *et al.* (2004) where 1ml of Aliquot from 10^3, 10^4, 10^5, 10^6 and 10^7 dilutions were transferred into duplicate Petri dishes. This was followed by pouring aseptically about 15ml of molten agar. The culture was then homogenized by swirling the plates and later allowed to solidify. The plates were incubated at 37°C for 2hrs. Following incubation, plates containing 30 – 300 colonies were selected and the colonies counted, averaged and multiplied by the inverse of the dilution factor.

Detection and Enumeration of Coliforms

This was carried out according to a method described by Atlas (1997). A set up consisting of 9 test tubes each containing 9ml lactose broth and an inverted Durham tube were autoclaved to expel air and to become sterilized. Inoculation was made from the serially diluted samples as follows: From the 1:10 dilution, 1ml of inoculum was transferred to each of the first three of the 9 test tubes containing 9ml of lactose broth. Then 1ml was also transferred from 1:100 dilution to each of the second set of 3 test tubes of lactose broth and finally 1ml of inoculum was transferred from 1:1000 dilution to each of the last 3 tubes. The 9 test tubes were later incubated at 37°C for 24hours. The tubes were later observed for gas production and the number of gas positive tubes was compared with the most probable (MPN) table to estimate the most probable number of coliforms per gram of sample.

Procedure for Indole Test

The Indole test was carried out by preparing a Tryptone broth which was drawn in to test tubes, sterilized by autoclaving, inoculated with loopful of suspension and subsequently incubated at 37° C for 24 hours.

Following incubation, 3 drops of xylene was added in tubes, shaken vigorously and the tubes kept for the two layers to get separated. One millilitre of Kovac's reagent was added, and the formation of pink colour ring was observed. Positive Indole test was inferred by the formation of pink colour ring.

Procedure for Methyl Red Test

The Methyl red test was carried out by preparing Glucose phosphate broth, dispensed in test tubes, sterilized by autoclaving and the test tubes were inoculated with test culture and incubated at 37°C for 24 hours. Following incubation, five drops of methyl-red indicator was added to the medium to detect 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 then vigorously shaken, every 30 seconds where a positive reaction was indicated by the development of a pink color, which turns red in 1–2 hours, after vigorous shaking. The vigorous shaking was done to achieve complete aeration.

Procedure for Citrate Utilization Test

The 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 later held in slanted position, inoculated with the given bacterial culture and incubated at 37°C for 24 hrs. Growth during incubation results in the color change of the media from green to blue confirming a positive test.

Procedure for Catalase Test

The catalase test was carried out by spreading the bacteria on an agar plate followed by incubation of the plate over night at 37°C for 24 hrs. Portion of the bacteria from one colony was picked and placed on a clean and grease free microscope slide using sterile inoculating loop. One drop of 3% H_2O_2 was added to the bacteria and gas formation (O_2) in the form of bubbles, was observed showing that the bacterium is catalase positive.

Procedure for Coagulase Test

Coagulase test was carried out by suspending one colony from the suspected pure culture in 0.5 ml of human plasma and incubated at 37°C for 18hrs. The test was read twice, first after 4 hrs and finally after 24hrs. Positive reaction shows a stable plasma coagulate which do not dissolve upon stirring.

Procedure for Triple Sugar Iron Agar Test (TSI)

The Triple Sugar Iron Agar Test (TSI) was carried out by using a straight sterile wire loop to touch a well isolated colony. The TSI was inoculated by first stabbing through the centre of the medium to the bottom of the tube and subsequent streaking the surface of the slant. The tube was then incubated at 35°C in ambient air for 18 to 24 hours with the cap loosed.A black precipitate in the butt indicates production of H_2S. H_2S produced reacts with ferric salt to produce black precipitate of ferrous sulfide. Bubbles or cracks in the tube indicate the production of CO_2 or H_2. The broth medium was inoculated with a loopful of a pure culture of the test organism and the surface of the agar slant was also streaked with the test organism. The test tube was incubated at 35°C in ambient air for 18 to 24 hours while leaving the cap loosely bound. Organisms that hydrolyze urea rapidly (e.g. *Proteus* spp) may produce positive reactions within 1 or 2 hours; less active species (e.g. *Klebsiella* spp) may require 3 or more days. In routine diagnostic laboratories the Urease test result is read within 24 hours. Color change of the slant from light orange to magenta indicates Urease enzyme production by the organism. If organism do not produce Urease the agar slant and butt remain light orange (medium retains original color).

RESULTS AND DISCUSSION

The result of the microbial qualities of the *kilishi* samples are presented in Tables 1 and 2. The result indicated that, sampling point C had the highest aerobic mesophilic bacterial counts, 5.43 x 10^5cfu/g followed by sampling point B, 4.83 x 10^5cfu/g, while point A had the least aerobic plate counts of 3.92x 10^5cfu/g. Therefore, the total aerobic plate count (cfu/g) reflects that there were significant variations among the *kilishi* samples which suggests that some of the spices used in the *kilishi* production might played a key role in inhibiting the growth and multiplication of some microbes.

This is in agreement with Inusa and Sa'id (2017), Fonkem *et al*. (2010) and Shamsuddeen (2009). Similarly, the degree of meat spoilage is usually influenced in part by the microbial load at the beginning of production, packaging and handling of the finished product (Inusa and Sa'id, 2017).

Kilishi samples from sampling point C had the highest total coliform count, 50 MPN/g followed by sampling point B, 37 MPN/g while sampling point A had the least, 30 MPN/g. Thus, *Kilishi* samples retailed in the study area were found to be contaminated by coliforms. Contamination of ready-to-eat meat products has been reported by many researchers (Chukwu and Imodiboh, 2009; Fonkem *et al.*, 2010; Salihu *et al.*, 2010; Iheagwara and Okonkwo, 2016). However, this microbial contamination could be traced to unhygienic processing and low level of sanitation.

Occurrence of bacteria revealed that, *E. Coli* (100%), *Klebsiella* species (100%) and *Staphylococcus aureus* (100%) were present in all the samples of *Kilishi* from the three locations, while *Enterobacter* (33.3%) was present in *Kilishi* sampled at Kunchi town (Table 3). Similarly, microbial loads are higher at Kunchi town (100%) compared to Malikawa Garu and Shuwaki (75%), each. This might not be unconnected with contamination during transit from the production sites (Malikawa Garu and Shuwaki) to the retailing centres at Kunchi town. The occurrence of *Enterobacter*, in addition to the other microorganisms, in Kunchi town is an indication of poor handling, packaging and transportation of the product before reaching or during the retailing at Kunchi town, since most of the *Kilishi* retailed at Kunchi is being processed at Malikawa Garu and Shuwaki. This finding concords with the findings of several other researchers such as Edema *et al*. (2008), Shamsuddeen (2009), Fonkem *et al*. (2010), Daminabo *et al*. (2013), Odey *et al*. (2013), Okonko *et al*. (2013), Inusa and Sa'id (2017). *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus spp*, *Salmonellaspp*, *Bacillusspp*, *Pseudomonasspp* and *Proteusspp*. were isolated from selected *Kilishi* samples on sale at Calabar, Cross Rivers State, Nigeria (Odey*et al.*, 2013). Similarly, Okonko *et al*. (2013) isolated *Bacillus* species, *Staphylococcus aureus* and *Escherichia coli* in *Kilishi* samples from Port Harcourt, Rivers State. Moreover, in addition to *Bacillus cereus*, Edema *et al*. (2008) isolated, *Staphylococcus aureus* and *Salmonella*s pp from a study on *Kilishi* samples retailed at some selected cities in Southwestern Nigeria. In another study, Fonkem *et al*. (2010) isolated *E. coli* and *Staphylococcus aureus* from Cameroonian *Kilishi* while Shamsuddeen (2009) reported the presence of *E. coli*, *Salmonella* species, *Staphylococci* and *Clostridium perfringens* in spices used in the production of *Kilishi*.

Many studies reported that, commercial *Kilishi* samples from FCT Abuja (Daminabo *et al*. 2013), Port Harcourt (Okonkwo *et al.*, 2013) and Calabar (Odey *et al.*, 2013) had better microbial quality than that obtained from Kano. Abdullahi *et al*. (2016) reasoned that, differences in meat handling practices, ingredients, processing methods and variation in environmental factors may influence the microbial load of meat products. The International Commission on Microbiological Specifications for Foods (ICMSF, 1996) reported the limits for total aerobic bacterial and fungal counts to be in the order of $\leq 10^3$ as acceptable and 10^4 to 10^5 tolerable for ready to eat foods. Similarly, London Health Protection Agency, (LHPA, 2009) put $<10^6$cfu/g as satisfactory limit, and 10^6 to $<10^7$cfu/g as acceptable range. Range of contamination for *Enterobactericeae* was reported to be 10^2 - 10^4cfu/g (Shamsuddeen, 2015). However, the results of this study shows higher microbial count when compared to the acceptable limit, indicating that, the *Kilishi* products studied was of poor micobiological quality. This might be as a result of unhygienic practices during processing, handling and retailing. Ananias and Roland (2017) reported similar view in a study on microbial contamination of ready to eat meats vended in highway markets in Uganda. In a one year study conducted on the microbiological quality of *Kilishi* in Northern Cameroon results indicated that the total bacterial, mould and yeast counts (cfu/g) were lower than the recommended acceptable limit for the total viable bacterial counts of micro-organisms in meat at the point of consumption and that the quality of *Kilishi* was greatly affected by the season and site of production (Fonkem *et al.*, 2010). Thus, the levels of microbiological contamination revealed in the present study could be of public health concern as conditions favouring growth and proliferation of microbes prevails in most of the retail outlets.

Table 1: Total Aerobic Count for *Kilishi* in Kunchi Local Government Area

Location	Mean APC ± STD (cfu/g)	P – value
Malikawa Garu	$3.92 \times 10^5 \pm 36074.00$	0.009
Shuwaki	$4.83 \times 10^5 \pm 21778.43$	
Kunchi Town	$5.43 \times 10^5 \pm 151838.10$	

Table 2: Total Coliform Count for *Kilishi* in Kunchi Local Government Area

Location	Mean TCC ± STD (MPN/g)	P – value
Malikawa Garu	30 ± 3.5355	0.040
Shuwaki	37 ± 2.8868	
Kunchi Town	50 ± 11.6762	

Table 3: Occurrence of Bacteria in *Kilishi* Sampled at Kunchi Local Government Area

Location	*Escherichia coli*	*Enterobacter*	*Klebsiella*	*Staphylococcus*	Number (%)
Malikawa Garu	+	-	+	+	3(75%)
Shuwaki	+	-	+	+	3(75%)
Kunchi Town	+	+	+	+	4(100%)
Number (%)	3(100%)	1(33.3%)	3(100%)	3(100%)	

CONCLUSION AND RECOMMENDATIONS

It has been established that *Kilishi* product processed and retailed at Kunchi Local Government may be contaminated with microorganisms where APCs and TCCs were found to exceed the minimum acceptable limit. The presence of *E. coli* and other members of the *Enterobacteriaceae* signify poor microbiological quality of the product which could lead to unhealthy implications on consumption. It is therefore suggested that, good production practices, packaging, storage and transportation of *Kilishi* products should be observed.

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