MICROBIOLOGICAL QUALITY OF SPICE USED IN THE PRODUCTION OF KILISHI A TRADITIONALLY DRIED AND GRILLED MEAT PRODUCT

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ABSTRACT
Study was carried out on the microbiological quality of the spice mix used in the production of Kilishi. Twenty samples were analyzed. The analyses included; aerobic mesophilic bacterial count, staphylococcal count, fungal count, coliform count, detection of E. coli, Salmonella spp and Clostridium perfringens. The result of the analysis showed that the samples had a mean aerobic plate count, staphylococcal count and fungal count of 2.96×10^8, 1.73×10^7, and 1.04×10^5 cfu/g respectively. Coliform MPN of >2400/g. E. coli was detected in one of the samples, Salmonella spp one sample and Clostridium perfringens from four samples. The counts obtained were higher than the maximum acceptable levels provided by the Food and Agriculture organization (FAO) of the United Nations. It is therefore recommended that strict hygienic measures should be observed during the mixing of the spice in order to reduce the microbial load to an acceptable level.

Key words: Microbiological, Quality, spice, kilishi.

INTRODUCTION

Kilishi is a sun dried spiced and grilled meat snack that can be kept for considerably long time without getting spoiled due to its dry nature. Application of spice is one of the most important stages during the production of *kilishi* because it is a critical control point (Shamsuddeen and Ameh, 2008).

Spices could be defined as the natural vegetable products or mixtures thereof, without any extraneous matter that is used for flavouring, seasoning and imparting aroma to foods International Standards Organization (ISO, 1972). Spices like other food substances, may carry some bacteria, yeasts, moulds spores and even some insects. The predominant flora is generally composed of aerobic spore and non spore forming bacteria, indicator organisms and some pathogens may also be found International Commission on Microbiological Specifications for Foods (ICMSF, 1986). Coliforms were isolated and characterized to be *E. coli, Klebsiella spp, Pectobacterium spp* and *Enterobacter* (Patel *et. al.,* 1976).

According to Frazier and Westhoff (2006), spices do not have a marked bacteriostatic effect in the concentrations used in meat products and they may even serve as source of contamination of processed product. Occurrence of microorganisms that are potentially pathogenic in spices used in *suya* preparation is considered as major cause of gastrointestinal disturbances resulting from the consumption of suya in Nigeria (Ejeikwu and Ogbonna, 1998). According to Price and Schweigert, (1971), unless spices are treated to reduce their microbial content, they may add high numbers and undesirable kind of organisms to food in which they are used.

Spice ingredients are thought to have some antimicrobial activities, and yet meat treated with spices have high microbial load (Shamsuddeen and Ameh, 2008). It is for this reason therefore, this research was set up with the aim of investigating microbiological quality of the spice used in the production of *kilishi* so that recommendation would be made based on the results obtained.

METHODOLOGY

Sample collection

Samples of spice mixture for *kilishi* production were collected from Dandalin Fagge at Fagge Local Government Area of Kano State. The mixture is composed of ginger, cloves, West African black pepper, hot pepper, groundnut, salt and seasoning. Twenty (20) Samples were collected using a sterile beaker, on different occasions and sample was taken to the laboratory immediately for analysis.

Sample Preparation and Serial Dilution

The sample preparation was carried out according to the method described by FAO (1979). In this method, 25g of sample (spice) was weighed and homogenized by blending in 225ml peptone water at 15,000-20,000 rpm. This was labeled as 1:10 dilution which is also the stock or the homogenate. This was further serially diluted to $1:10^7$.

Total Aerobic Plate Count

This was carried out according to the method of Adullahi *et. al.,* (2004). One milliliter (1ml) of inoculum from 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions were transferred into duplicate Petri dishes which were labeled accordingly.

This was followed by pouring aseptically about 20-25ml of molten nutrient agar. The inoculated was mixed by swirling the plates and later allowed to solidify. The plates were incubated at 37°C for 24hrs. After incubation, plates containing 30-300 colonies were selected and the colonies counted and recorded. The average was taken and the number obtained was multiplied by inverse of the dilution factor. This gave the number of colony forming units per gram of each sample (cfu/g).

Enumeration and Detection of *Staphylococcus aureus*

This was carried out according to Abdullahi *et. al.*, (2004). A quanty (0.25ml) of inocula from 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions were transferred into duplicate Petri dishes which were labeled accordingly. This was followed by pouring aseptically about 20-25ml of molten Baird Parker agar (Biotek). The plates were incubated at 37°C for 24hrs. Plates containing 30-300 black colonies were selected and the colonies counted. The average was taken and the number obtained was multiplied by four and then by the inverse of the dilution factor. This gave the number of colony forming units per gram of a sample (cfu/g). Plates of mannitol salt agar were inoculated and incubated at 35ºC for 24hrs. Following incubation, mannitol fermenting organisms which showed a yellow zone surrounding their growth were isolated onto agar slants for biochemical tests.

Enumeration and Identification of Yeast and Mould

This was carried out according to the method of FAO (1979). One millilitre (1ml) of inocula from 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions were transferred into duplicate petri dishes which were labeled accordingly. This was followed by pouring aseptically about 15-20ml of molten malt extract agar. The inoculated medium was mixed by swirling the plates and later allowed to solidify. The plates were incubated at 20-25ºC for 5 days. After incubation, plates containing 30-300 colonies were selected and the colonies counted. The average was taken and the number obtained was multiplied by the inverse of the dilution factor. This gave the number of colony forming units per gram of a sample (cfu/g).

Enumeration and Detection of Coliforms

This was carried out according to method described by Atlas, (1997). In this method, a set up consisting of 9 test tubes each containing 9ml of lactose broth and an inverted Durham tube, were autoclaved to sterilize and expel air. 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 also was transferred from 1:100 dilution to each of the second set of three test tubes of lactose broth and finally 1ml of inoculum was transferred from 1:1000 dilution to each of the last three tubes. All the 9 test tubes were incubated at 37ºC for 24 hours and another 24 hours in the absence of gas (presumptive test). Following 24 hrs of incubation the tubes were observed for gas production and the number of gas positive tubes was compared with the most probable number (MPN) table to estimate the most probable number of coliforms per gram of sample. 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. Following incubation, colonies which formed bluish black colour with green metallic sheen, and reddish colonies were noted and isolated on agar slants. This is called the confirmatory test. Also colonies showing metallic sheen on EMB, were sub cultured into tubes of lactose broth and incubated at 37ºC. The tubes were observed after 24hrs., for gas production. This is the completed test for fecal coliforms.

Detection of *Clostrdium perfringens*

This was carried out according to method described by Cheesbrough (2000). The homogenate was streaked onto neomycin blood agar and incubated anaerobically at 37ºC for 24 hours. Following anaerobic incubation, the plates were checked for large β-haemolytic colonies.

Detection of *Salmonella*

This was carried out according to FAO (1979). The homogenate was incubated at 37ºC for 20hrs. After incubation, 10ml was transferred into 100ml selenite cystine medium and incubated at 37ºC for 24 hours (enrichment). A loopfull from the enrichment medium was streaked onto brilliant green agar (BGA) plates and incubated at 37ºC for 24-48hrs. The colonies were Gram-stained and observed for motility.

RESULTS

The result of the study is presented in Table 1. The spice samples analyzed had aerobic plate count, staphylococcal count and fungal count of 2.96×10^8 cfu/g, 1.73×10^7 cfu/g and 1.04×10^5 cfu/g respectively. The coliform count was >2400 for each sample. *E. coli* was detected in one sample, *Salmonella* spp, one sample and *Clostridium perfringens* was detected in four samples.

Table 1: Microbiological analysis of spice samples

S/No.	APC (cfu/g)	SC (cfu/g)	FC (cfu/g)	CC (MPN/g)	*E. coli*	*Salmonella*	*C. perf*
1	TNTC	1.72×10^6	1.03×10^5	>2400	-	-	-
2	TNTC	1.62×10^7	1.05×10^4	>2400	-	-	+
3	2.11×10^9	2.20×10^7	3.30×10^4	>2400	-	-	-
4	2.21×10^8	1.12×10^7	1.04×10^5	>2400	-	-	-
5	1.11×10^9	1.60×10^6	1.01×10^5	>2400	-	-	-
6	4.90×10^8	3.30×10^7	1.03×10^5	>2400	-	-	+
7	TNTC	1.11×10^7	5.50×10^4	>2400	-	-	-
8	2.22×10^9	7.10×10^6	1.72×10^5	>2400	-	-	-
9	TNTC	1.19×10^7	1.05×10^5	>2400	-	-	-
10	1.15×10^9	1.10×10^7	1.43×10^5	>2400	-	-	-
11	TNTC	1.72×10^7	1.60×10^5	>2400	-	-	+
12	TNTC	1.71×10^7	1.03×10^5	>2400	-	-	-
13	TNTC	2.11×10^7	1.93×10^4	>2400	-	-	-
14	9.60×10^8	1.13×10^7	7.00×10^4	>2400	-		-
15	TNTC	2.11×10^6	1.00×10^5	>2400	-	-	-
16	5.60×10^9	1.17×10^7	1.11×10^5	>2400	+	+	-
17	TNTC	1.02×10^7	1.04×10^5	>2400	-	-	-
18	2.21×10^9	1.72×10^7	1.23×10^5	>2400	-	-	-
19	3.90×10^9	1.72×10^7	1.10×10^5	>2400	-	-	-
20	1.55 E10	9.40×10^6	2.50×10^5	>2400	-	-	+
Means	**2.96×10^9**	**1.73×10^7**	**1.04×10^5**	**>2400**			

Key:
+ = Detected, **- =** Not detected, APC = Aerobic plate count, SC= Staphylococcal count, FC=Fungal count, CC= Coliform count, TNTC= Too numerous to count at all the dilutions made.
cfu/g   = colony forming unit per gram.
MPN/g = Most probable number/gram.

DISCUSSION

The mean values of the obtained from the aerobic mesophylic count, staphylococcal and fungal counts from the spice used in the production of *kilishi* were high. This is because aerobic plate count of 10^8cfu/g, staphylococcal count of 10^7cfu/g and fungal count of 10^5cfu/g are all high. These values are higher than the maximum acceptable levels provided by the Food and Agriculture organization of the United Nations according to which the aerobic plate and fungal counts of spices should not be greater than 10^6 and 10^4cfu/g respectively.

The high staphylococcal count is also a point of concern since the growth of *Staphylococcus aureus* to a population of 10^5 cfu/g is considered necessary for the production of 1µg of enterotoxin sufficient to cause intoxication if such food is consumed, However ICMSF (1978) indicated that 10^6 cells of *S. aureus*/gram is required to present the risk of intoxication. Another point of deviation from FAO standard in the spice is the high coliform count and the presence of *E. coli* in particular, which is an indication of improper and inadequate hygienic practices. Shamsuddeen and Ameh, (2008) also reported the presence of high bacterial load as well as presence of coagulase positive *Staphylococci* and *E. coli* in *kilishi* from Kano metropolis.

The high bacterial load in the spices is an indication of unhygienic practices during their preparation. Contaminating organisms might have come from hands of handlers (Bukar et al., 2009), the utensils from air and even from the spice ingredients themselves because according to Frazier and Westhoff (2006), spices may even serve as source of contamination of processed product. Price and Schweigert, (1971), reported that unless spices are treated to reduce their microbial content, they may add high numbers and undesirable kind of organisms to food in which they are used.

Conclusion and Recommendations

The microbial loads of spice used in *kilishi* production is high, there was also in the spice indicator organisms and organisms that are potentially pathogenic. It could therefore be recommended that;
- Spices should be produced under strict hygienic measures.
- The spice should be subjected to treatment that would reduce their microbial load. This is to avoid the introduction of undesirable kinds of organisms that might bring about spoilage and contamination.

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