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Quality variation of Kilishi from Different Locations in Nigeria

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Abstract

Kilishi is an intermediate moisture meat that has a suitable concentration of dissolved solids that binds the moisture in it sufficiently to inhibit the growth of spoilage organisms. Samples of kilishi were collected from three different locations [Abuja (FCT), Kaduna (Kaduna state) and Ibadan (Oyo state)], a fourth sample was prepared in the laboratory to serve as control in a completely randomized design. Samples were packaged in three different media viz; aluminum foil (AF), transparent polyvinyl chloride bags (PVC) and transparent plastic containers with lids (PC). 100g of kilishi samples was packaged into each medium and left at ambient temperature (30-38^0). The least moisture content of 6.98±0.65% was observed in the control, which differed significantly (P<0.05) from moisture contents of other samples. No difference (P>0.05) was observed for moisture between kilishi from Abuja and Kaduna. The mean crude protein content varied from 47.98±1.74 to 59.32±0.36%. All values reported differed significantly (P<0.05) from one another. For ether extract, the least value was 14.90±0.87%, while the highest was 15.75±0.36%. The PVC packaging contained the lowest microbial load of 3.18±0.01cfu/g for Kaduna kilishi at the onset of storage followed closely by the Ibadan kilishi. Three bacteria spp were isolated from the kilishi samples from the different locations. The isolates were identified as Bacillus subtilis, Bacillus pumilus, and Staphylococcus spp.

Keywords: Kilishi, chemical composition, packaging, microbial count, salt level.

Introduction

Kilishi is an intermediate moisture meat. It is a traditional sun-ried Nigerian and Sahelian African (Niger Republic) meat product whose origin is lost in antiquity. *Kilishi* is principally processed from beef, although goat and sheep meat can also be used (1 and 2). In recent time pork has been introduced as possible meat for *kilishi* production (3). The product came about as a means of preserving meat in the absence of facilities for refrigerated storage by the early Fulani and Hausa herdsmen. Some Northern states which are main processors of *kilishi* include: Borno, Kano, Sokoto, Kaduna and Bauchi. This is made possible because the weather is favourable and consumer demand is high. The product has gained popularity even today in all major urban as well as rural centres, particularly in the Northern parts of Nigeria where it is sold in the streets and in some supermarkets - the only traditional Nigerian meat product to attain the latter status (4).

Kilishi is a ready-to-eat, convenient meat product; it appears to have excellent shelf stability at room temperature, making handling and marketing of the product convenient for consumers and retailers alike (4). It is noteworthy however that the quality of *kilishi* produced by the traditional processors varies from one

producer to the other, and from one batch to another from the same producer due to the lack of standardized ingredient mix and unit operation that would ensure consistent product quality (5). There are differences in the quantity of products from one location to another due to the wide range of ingredients used. This study was aimed at comparing the proximate composition and the shelf life of representative samples packed in various media using the microbial profile as the yardstick.

Materials and Methods

Collection of samples

Samples of *kilishi* were collected from three different *kilishi* producing states - Abuja (FCT), Kaduna (Kaduna state) and Ibadan (Oyo state), a fourth sample was prepared in the laboratory to serve as control in a completely randomized design. The commercial samples (usually packaged in newspaper print or the conventional cleaned cement paper) were put in sterile plastic containers from the point of purchase and brought to the laboratory as soon as possible in readiness for use on the following day. The *kilishi* samples from each town were obtained from at least three different locations, then mixed together to give a representative sample.

Packaging of samples for storage

Individual samples collected in sterile plastic containers were left at room temperature in the laboratory overnight. 50g was taken from each sample for the proximate analysis and microbial studies. The remaining samples were packaged in three different media to observe their shelf life. These media were aluminum foil (AF) transparent polyvinyl chloride bags (PVC) and transparent plastic containers with lids (PC). 100g of *kilishi* samples was packaged into each medium and left at ambient temperature (30-38^{0}C). The transparency of the PVC and PC containers allowed for easy observation. This was not obtainable for the foil (PF) packaged samples. The *kilishi* were stored for a period of 18 months at room temperature in these storage media with samples removed for proximate analysis and microbial evaluation after 12 months and at 18 months.

Microbial Analysis

Five different culture media were used to carry out the bacteriological and mycological analysis. These were the plate count agar (PCA), which gives an estimation of microbial count, MacConkey agar (MA) for coliform bacteria, yeast extract agar (YEA) for yeast nutrient agar (NA) for general microbial analysis and Potato dextrose agar (PDA) for moulds. They were incubated for 2-3 days except for yeast and moulds that were incubated for 5days.

Isolation techniques – serial dilutions

Isolations were made from the samples using the serial dilution methods of Meynelle and Meynelle (6).

Morphological studies

Colonies, which developed after incubation were examined for cultural features such as elevation, size, surface form, degree of growth, opacity, edge, consistency and pigmentation. Pure cultures of the associated microorganism

were obtained by repeated streaking on nutrient agar plates for bacterial and fungal isolates. Cellular characteristics of the pure culture of each isolated microorganism were examined under the microscope using the oil immersion objective after gram staining (7).

Biochemical characterization of the isolates

Bacterial Isolates: Various tests were carried out on the bacterial isolates for possible identification. In each case a fresh 18-24hrs old culture was used for every biochemical test.

Bacteriological and mycological examination of samples

Isolates were observed macroscopically and microscopically. For macroscopic examination of fungi isolates, the isolates were examined physically on plates based on colour and hyphae formation, while for microscopic examination, a small portion of the mycelium of the fungi isolated was picked from the culture plate with sterile inoculating needle and placed on a macroscopic slide. This was stained with lactophenol cotton blue, the slide was then viewed carefully under a light microscope of x40 objective (8).

Chemical Analysis

The proximate composition of meat samples was carried out by the methods of the Association of Official Analytical Chemists (9). This was done at the onset, at 12 months and at the end of storage for 18 months.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using the SPSS package and significance between means were separated using Duncan's multiple range test (10).

Results and Discussion

Chemical composition of *kilishi*

Table 1 shows the mean proximate values of *kilishi* from the three different locations and that of the control. The least moisture content of 6.98±0.65% was observed in the control and differed significantly from moisture contents in other samples. No difference ($P>0.05$) was observed for moisture between *kilishi* from Abuja and Kaduna, although *kilishi* sample from Kaduna had a higher moisture content (11.01±0.74%.). The mean crude protein content varied from 47.98±1.74 to 59.32±0.36%. All values reported differed significantly from one another. For ether extract no significant differences ($P>0.05$) were obtained for all samples. The least value was 14.90±0.87% while the highest was 15.75±0.36%. Crude fibre values reported showed a significant difference ($P<0.05$) between Ibadan (2.70±0.14%) and that of Abuja (3.57±0.40%), Kaduna (3.36±0.18%) and the control (3.47±0.26%).

Kilishi is an intermediate moisture meat that has a suitable concentration of dissolved solids that binds the moisture in it sufficiently to inhibit the growth of spoilage organisms. The control *kilishi* exhibited the least moisture content relative to the other locations (Table 1). Values obtained are comparable to that of other workers. Egbunike and Okubanjo (11) reported a moisture content range of 8.16-11.14% in their study on comparing oven-dried and sun-dried *kilishi*. Jones *et al.* (12) gave the moisture content of

traditionally prepared *kilishi* as 6.92% two days post-production.

The ratio of moisture to ash (M/A) as reported by (13) is an indication of the relative amount of salt incorporated within charqui - an intermediate moisture meat. They reported values of 1.3 during drying and 1.7 for packaged products. The study gave an M/A range of 0.8-1.26 for finished *kilishi* product (Table 4). This is because the salt concentration inside the product is similar to that of the external surface, unlike charqui where the internal salt concentration differs from the external.

Torres *et al.* (13) in their study of indirect estimation of the water activity (a_w) of charqui noted a strong relationship between moisture, protein and salt contents; they reported that it is possible to use the ash/protein ratio to determine indirectly the a_w value. They reported stability in the ratio after the value of 0.4 was reached, which corresponded to values of 0.70-0.75 a_w. The values obtained in this study however did not seem to follow this trend; the A/P ratios obtained were as low as 0.15-0.18 (Table 1).

The ash content is an indication of the mineral content of the product. The ash values of Abuja product significantly differed from that of Ibadan, while that of Kaduna and the control did not differ significantly from that of Kaduna or Abuja. Igene *et al.* (4) reported an ash value of 9.6% for traditionally processed *kilishi*.

The moisture content of fresh muscle is about 75%, the crude protein is 16-22%, the lipid content is 1.5-13%, while the carbohydrate content varies from as little as 0.5-1.3% (15). Igene *et al.* (4) obtained 50.2% protein content for traditionally processed *kilishi*. Egbunike and Okubanjo (11) reported protein values of 68.06-71.75% for sun-dried and oven-dried *kilishi*. The protein content of *kilishi* from the different locations fell within this range (Table 1). The high protein value of *kilishi* makes it a food or snack very rich in protein, the spices and condiments added in its processing must have added to this significant increase.

Table 2 gives the microbial load of *kilishi* from the different locations, under three different storage regimes, and different packaging. No visible microbial growth were detected for the control samples packaged in plastic containers and polyvinyl chloride bag (freezer bags), throughout the storage period while slight increases were observed in *kilishi* samples packed in foil paper. Significant increases above the baseline loads were observed in *kilishi* from the three different locations and packaged in the three different media over the evaluation period.

The foil packaged *kilishi* samples had the highest mean microbial load at all storage times from all the locations. *Kilishi* from Abuja and Ibadan had increasing bacterial count from the first week of storage to the 72nd week. The Kaduna *kilishi* experienced a drop in the microbial count at 48 weeks of storage (5.11±0.01) and rose to 6.36±0.1 cfu/g count at 72 weeks of storage. The laboratory prepared *kilishi* had a low count of 3.04±0.01 cfu/g at the onset of storage, it had 100% hike (6.32±0.1) cfu/g in the count at 48 weeks and dropped to 4.05±0.1 cfu/g at 72 weeks of storage.

The polyvinyl chloride bags (PVC) packaging recorded the lowest microbial load of 3.18±0.01cfu/g for Kaduna *kilishi*

at the onset of storage, followed closely by the Ibadan *kilishi*. The Abuja *kilishi* had the highest count of 3.85±0.01 cfu/g at this time of storage. A sharp increase in the microbial count was noticed at week 48 of storage for Abuja, Kaduna and Ibadan *kilishi*. While week 72 gave a slight increase in the bacterial load for Abuja and Kaduna, Ibadan *kilishi* had a sharp decline in bacterial count. No count was observed for the control *kilishi* at the three storage times.

Results for the plastic packaged *kilishi* showed that at week 1, Abuja *kilishi* had the least microbial count of 3.18±0.01 cfu/g, while *kilishi* from Ibadan recorded the highest of 4.23±0.01 cfu/g for the count. Both Abuja and Kaduna *kilishi* had the same microbial count of 4.05±0.01 cfu/g while a slight increase was observed at week 72 for Abuja and Kaduna *kilishi* samples. For all the weeks the *kilishi* from Ibadan had the highest microbial load, giving 6.08±0.01 cfu/g at week72.

It can be deduced from the present study that *kilishi* samples from the different locations varied in microbial load as a result of the packaging method used and the length of time for which it was stored thus resulting in varying qualities (Table 2).

Table 3 gives the summary of microorganisms associated with different *kilishi* samples. The *Bacillus* spp. of bacteria seemed to be the most recurring in all the samples. The control *kilishi* had no *Staphylococcus spp* found in them. Out of the isolates in the study, eight were randomly selected to test for salt tolerance of some of these organisms. Results presented in Table 4 showed Bacillus spp. and *Proteus* spp. to tolerate 6% saline concentration, but no survival at higher concentration. Streptococci and Staphylococcus on the other hand were able to grow at concentrations as high as 10% (Table 4).

In the present study the Bacillus specie was predominant in several samples, an indication of their ubiquitous nature. The soil is an important source of Bacillus species (14). Therefore, considering the slaughtering and handling of meat which expose it to the soil and air before processing, and further air drying on floor or mats predisposes *kilishi* to have contact with this bacteria.

Some of the toxigenic cocci are very salt tolerant, growing in sodium chloride solutions that approach saturation, and also tolerate nitrites fairly well and therefore can grow in curing solutions and on curing and cured meats if other environmental conditions are favourable. They are also fairly tolerant of dissolved sugar (14). The present study has shown that streptococci and staphylococci spp tolerate salt concentration as high as 10%, proteus spp on the other hand could not survive at above 6% salt concentration.

Conclusion

Kilishi from different locations varied in microbial load as a result of the packaging method used. Hygenic handling of fresh and processed meat could considerably reduce the load of microbes. PVC and PC materials are better packaging materials than the foil and cement paper if the products are not to be consumed immediately.

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Table 1: Mean proximate analysis of *kilishi* from different locations

Chemical Composition	Location			
	Abuja	Kaduna	Ibadan	Control
Moisture	9.56±1.39[b]	11.01±0.74[c]	10.73±1.73[c]	6.98±0.65[a]
Crude protein	47.98±1.74[a]	52.17±1.66[c]	50.52±0.99[b]	59.32±0.89[d]
Ether Extract	15.45±1.01	14.90±0.87	15.02±0.77	15.60±1.03
Crude fibre	3.57±0.40[b]	3.36±0.18[b]	2.70±0.14[a]	3.47±0.26[b]
Ash	8.18±0.99[a]	8.71±0.13[b]	9.35±0.19[c]	8.70±0.34[b]
Moisture: Ash	1.17±0.02[b]	1.26±0.01[a]	1.15±0.02[b]	0.80±0.05[c]
Ash: Protein	0.17±0.04	0 .17±0.16	0.18±0.02	0.15±0.05

[a,b,c,d] Means on the same row with different superscripts are significantly (P<0.05) different. Means of triplicate determinations with standard deviations.

Table 2: Mean (x) and standard deviation (SD) of colony counts of *kilishi* as affected by packaging method, storage time and location of purchase (log_{10} c.f.u./g).

Packaging method	Storage time (Week)	Location			
		Abuja	Kaduna	Ibadan	Control
Foil	1	4.79 ± 0.01	5.49 ± 0.01	5.32 ± 0.01	3.04 ± 0.01
	48	5.18 ± 0.01	5.11 ± 0.01	6.11 ± 0.01	6.32 ± 0.01
	72	6.45 ± 0.01	6.36 ± 0.01	6.49 ± 0.01	4.05 ± 0.01
PVC	1	3.85 ± 0.01	3.18 ± 0.01	3.20 ± 0.01	0.00
	48	4.70 ± 0.01	4.17 ± 0.01	4.17 ± 0.01	0.00
	72	4.78 ± 0.01	4.23 ± 0.01	2.28 ± 0.01	0.00
Plastic	1	3.18 ± 0.01	3.32 ± 0.01	4.23 ± 0.01	0.00
	48	4.05 ± 0.01	4.05 ± 0.01	5.75 ± 0.01	0.00
	72	4.04 ± 0.01	4.17 ± 0.01	6.08 ± 0.01	0.00

PVC – Polyvinyl chloride bags

Table 3: A summary of Microorganisms Associated with Different Samples

Samples	Microorganism
S_1 Abuja	*Bacillus pumilus, Staphylococcus aureus, Mucor spp.*
S_2 Ibadan	*Bacillus subtilis, Staphylococcus aureus, Aspergillus fumigatus*
S_3 Kaduna	*Staphylococcus aureus*
S_4 Control	*Bacillus* spp., *Aspergillus niger*

Table 4: Salt tolerance of some organisms isolated

	NaCl Concentration		
Isolate	6%	8%	10%
Bacillus licheniformis	+ve	-ve	-ve
B. Subtilis	+ve	-ve	-ve
B. Pumilus	-ve	-ve	-ve
B. Subtilis	-ve	-ve	-ve
Streptococcus spp.	+ve	+ve	+ve
Staphylococcus aureus	+ve	+ve	+ve
B. Subtilis	-ve	-ve	-ve
Proteus spp.	+ve	-ve	-ve

+ve- Growth
-ve- No growth