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Quality evaluation of *kilishi*, an intermediate moisture meat product sold in Zaria metropolis, Nigeria

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Target Audience: Meat Processors, Meat Products Consumers, Meat scientists, Extension Officers, Public Health Officials and Animal Scientists.

Abstract

This study was carried out to evaluate the chemical and microbial qualities of Kilishi sold in Zaria metropolis, Nigeria. A total of thirty (30) samples from three different locations (Sabon-gari-(Site I), Zaria city (Site II) and Samaru area (Site III) were randomly collected. Control samples of Kilishi were prepared in the Meat Laboratory of the Department of Animal Science, Ahmadu Bello University. All Kilishi samples were subjected to chemical analysis and microbiological examination-aerobic plate counts (APC), staphylococcal counts (SC), fungal counts (FC) and coliform counts (CC). Kilishi from Site II had significantly ($p<0.05$) higher moisture values (7.52%) than Kilishi from the control (5.65%), Site I (5.19%) and Site III (5.44%), fat and ash contents were significantly ($p<0.05$) higher in control (22.53% and 7.80% respectively) than Kilishi from other sites. Microbial counts were high in commercial Kilishi samples with mean APC of 4.1×10^5, Coliform counts of 3.0×10^4 and FC of 5.9×10^6 in Site I while Staphylococcal counts was 7.0×10^4 in Site III. The general evaluation of microbial species showed the presence of Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Klebsiella sp in commercial Kilishi which could pose high health risk to consumers. It is therefore advised that processors of Kilishi should imbibe good hygienic practices in order to improve the quality and reduce the risk of food borne illnesses while consuming this product.

Keywords: Quality, Kilishi, chemical evaluation, microbiological analysis

Description of Problem

In the tropics, meat spoils rapidly few hours after the onset of rigor mortis hence the need for meat to be preserved in order to maintain its quality. One of the ways of preserving meat is processing. Processing enhances meat quality as well as elongates shelf-life. Processed meat products are defined as those in which the properties of fresh meat must have been modified by the use of one or more procedures such as

grinding, addition of seasoning agents, alteration of colour or heat treatment (1). Various processing methods are employed in the preservation of meat and these include drying, salting, curing, incorporation of additives, refrigeration and freezing. Meat from cattle, sheep and goats and chicken can be processed into products like, *Kilishi*, *Tsire, Asun, Balangu*, which are commonly served or sold along streets, in club houses, at picnics, restaurant and within institutions (2). The microorganisms which are found to contaminate and cause spoilage of meat and meat products are bacteria, yeast and moulds. These organisms are introduced into meat by butchers and workmen, or through water and air in the dressing, cooling and cutting rooms or tables and even from the environment (3). The high ambient temperature, humidity, shortage of portable water and poor handling practices predisposes meat and meat products to massive microbial contamination which consequently lead to rapid deterioration and even poisoning (4). This research therefore was carried out to determine the qualities of *Kilishi* as obtainable by consumers from the open market through microorganism load evaluation.

Materials and Methods

Experimental Location: The study was conducted in the Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria. Zaria is located on the high plains of Northern Nigeria, 652.6m above the sea level, 950km away from the coast (112 031N 7Q 42E).

Experimental Materials: Samples were collected from three different locations in Zaria, Kaduna State: Sabon-Gari (Site I), Zaria-city (Site II) and Samaru (including Hanwa, Rada Kano and Paladan areas) (Site III). Each of the sampling sites was randomly chosen among all the *Kilishi* production sites in Zaria.

Collection of Materials: A total of thirty (30) *Kilishi* samples were randomly obtained from producers from the production sites for two days. Samples were aseptically introduced into pre-sterilized plastic packs and transported to the Meat science laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria for immediate analysis.

Laboratory production of *Kilishi*: Four (4) kg meat (semi-membranosus muscle) from the hindquarter of freshly slaughtered cow was bought from the local abattoir in Zaria metropolis. It was trimmed free of fat, nerves, blood vessels and excess connective tissues. The meat was cleaned and refrigerated for 2h before processing. It was cut into small chunks of about 50 to 100 grams, slicing was done along the fibre axis of the portion giving very thin slices of about 1 to 1.5mm thickness in sheets. Drying was done in two stages. The first stage of sun-drying was done for 4h on day one. The second stage was done after the dried meat was immersed into a bowl containing 2L of slurry of spices (Table 1) for 4h and turned at intervals. Drying was accomplished when the meat sheets became crispy flakes and brittle. The finished product was passed through a glowing fire for 2min to heat-seal the ingredients infused in the product. The product became ready for consumption after cooling at room

temperature.

Microbial Analysis

Plate count agar (PCA), Nutrient agar (NA), Mannitol Salt agar (MSA), Salmonella shigella agar (SSA), Eosin Methylene Blue agar (EMB) and Malt exact agar (MEA) were used to carry out bacteriological and mycological analysis of *Kilishi* samples at the Microbiology laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria. These were prepared according to manufacturer's instructions and aseptically distributed into sterile Petri dishes. 25grams of each of the meat samples were weighed and transferred into 225 ml of peptone water in a beaker. Serial dilution was prepared from 10^2 to 10^6 and plated. They were incubated at 37°C for 1-2 days except for yeasts and moulds that were incubated for 4 days.

Morphological Studies

Colonies, which developed after incubation were examined for cultural features such as elevation, size, surface form, degree of growth, opacity 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 pure culture of each isolated microorganism (bacterial) were examined under the microscope using the oil immersion x100 objective after gram staining.

Table 1: Ingredients composition for *Kilishi* Slurry

Ingredient	Quantity (%)
Groundnut paste *(Tunkusa)*	38.0
Ginger	2.50
Cloves	2.50
Black pepper	3.40
Red pepper	1.10
Sweet pepper	1.10
Alligator pepper	1.80
Onion	6.70
Garlic	0.50
African nutmeg	1.00
Curry	0.70
Salt	0.10
Maggi (Knorr®)	2.10
Sugar	2.50
Water (ml)	36.0
Total	**100.0**

Biochemical Characterisation of Isolates

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

Mycological Examination of Samples

Fungi and yeasts insolates were examined physically on plates based on colour and hyphae formation. The characteristics of the isolates were identified using methods described by (5) and (6).

Chemical Analysis

The chemical composition of samples of *Kilishi* from both control (produced in the laboratory) and sites (the different locations) were carried out following the procedures of (7).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) of (8). The Duncan multiple range test subjected treatment means to comparison.

Results and Discussion

Chemical composition of commercial and control *Kilishi*

The mean chemical analysis of *Kilishi* is shown in Table 2. The result shows that there were significant differences ($p<0.05$) between moisture, fat, ash and fibre in the *Kilishi* products however no difference was observed for protein and carbohydrate ($p<0.05$). No significant variation occurred in the moisture content of *Kilishi* in control (5.65%), site I (5.19%), and site III (5.44%). The Fat and Ash compositions had the same trend. The fat content of *Kilishi* produced in the laboratory (control) was significantly ($p<0.05$) higher (22.53%) compared to the commercial samples from site I (20.49%), site II (17.09%), and site II (21.88%). Similarly, the ash content of control was significantly ($p<0.05$) higher (7.80%) compared to the commercial samples from site I (7.24%), site II (5.55%), and Site III (7.73%). However, *Kilishi* from Site II had the least Fat (17.09%) and Ash (5.55%) contents. The Crude fibre of *Kilishi* from sites II and III were not significantly different from each other with mean value of 0.55%, while the highest significant value was observed in *Kilishi* from the control (0.58%). No significant difference was observed in crude protein content of *Kilishi* with mean values ranging from 48.19% to 51.80% and carbohydrates with mean values ranging from 13.16% to 18.90%.

The results obtained for this study were lower than those recorded in previous works on *Kilishi*. (9) recorded moisture percentage of 11.6% for Tunkusa *Kilishi* and 12.1% for groundnut flour *Kilishi*, (4) obtained a mean percentage of 12.4%, (10) recorded 13.73(±1.05)%, while (11) recorded a value of 8.99 (±0.23)% for traditional *Kilishi*. The difference could be due to the processing method which led to reduced water activity (a_w) of the product. Water activity is the term for the amount of free (not chemically or physically bound) water, which is available for the growth of microorganisms (12). The moisture content obtained in this study indicated that the products were well dried and thus prevented microbial spoilage. The range of values obtained for protein content was however in agreement with previous works on *Kilishi*. (13) reported a value of 50.02% for traditional *Kilishi* after roasting, (9) obtained 49.8% for *Tunkusa kilishi* and 51.4% for groundnut flour *Kilishi* while (11) recorded 62.33(±17.05)% for traditional *Kilishi*.

The major part of the protein in *Kilishi* is usually from the groundnut paste used (14). All other ingredients do contribute their quota of protein too, making *Kilishi* a food or snack very rich in protein (15).The ash content of fresh meat is about 1% on wet basis, processing however increases this level significantly. On dry weight basis, it contains 3.5% mineral components (16).

The high ash content values recorded in this study could be due to individual mineral levels of spices used in the slurry formulation for processing (11) combined with the ash contents of the meat sample used for production. Ash content of 7.83% for traditional prepared *Kilishi* prior to roasting was also reported by (13). High fat content levels were also observed in this study. (10) noted that *kilishi* is very high in lipid content on dry weight basis (25.23%), this consisting mostly of triglycerides. A study of traditional processing of *kilishi* gave a fat percentage of 17.8% (11) and (16) reported a fat content as high as 25.36±1.35%. Fat content values obtained could be due to the level of fat in the groundnut paste used in production of the *Kilishi* samples. Meat do not contain dietary fibre, however crude fibre was determined sinceelements that constitute the spices used in *Kilishi* production are of plant origin (9) reported crude fibre of 3.1% for *Tunkusa Kilishi* and 2.8% for groundnut flour *Kilishi* and (17) obtained values of 0.13±2.85 to 0.17±2.55% for *Kilishi* with different drying methods.

Table 2: Chemical Composition (%) of *Kilishi* samples

Treatments	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate
Control	5.65^{b}	50.57	22.53^{a}	7.80^{a}	0.58^{a}	13.25
Site I	5.19^{b}	48.19	20.49^{b}	7.24^{b}	0.33^{b}	18.90
Site II	7.52^{a}	51.47	17.09^{c}	5.55^{c}	0.55^{a}	17.88
Site III	5.44^{b}	51.80	21.88^{ab}	7.73^{ab}	0.55^{b}	13.16
SEM	0.11	0.94	0.27	0.10	0.02	1.06

abc Means bearing different superscripts within the same column differ significantly ($p<0.05$).
Site I = Sabon-gari. Site II =Zaria city. Site III =Samaru area. SEM= Standard Error of the mean.

The significant differences obtained could be due to the ingredient composition and varied rate of absorption and adsorption of the dried raw meat slices in the slurry. The substantial part of the carbohydrate contents of the products in this study could have been contributed by the ingredients in the slurry since they are of plant origin which is high in common sugars. Similar results were also obtained by (9) with 18.9% carbohydrate content obtained in *Tunkusa Kilishi* and 14.8% in groundnut flour *Kilishi*.

Microbial Counts of Commercial and Control *Kilishi*

The microbial counts of *Kilishi* products from the various sites and controls are presented in Table 3. Significant differences ($p<0.05$) were observed for aerobic plate counts (APC) and fungal counts (FC) with Site I having highest values of 4.1×10^{5} and 5.9×10^{6}, respectively. *Kilishi* from site III also had significantly higher values (7.0×10^{4}) for Staphylococcal counts (SC). However, no significant difference ($p>0.05$) was recorded for Coliforms counts (CC). Mean CC of 3.0×10^{4} which was the highest obtained in this study for the *Kilishi* samples were recorded for Site I with Site III having the lowest value (1.0×10^{4}). Site I recorded the highest mean FC value of 5.9×10^{6} while control had the least value of 2.0×10^{4}.

Table 3: Microbiological analysis of *Kilishi* samples (cfu/g)

Treatments	APC	SC	CC	FC
Control	2.0×10^{5b}	0.0	0.0	2.4×10^{4c}
Site I	4.1×10^{5a}	3.0×10^{4b}	3.0×10^{4}	5.9×10^{6a}
Site II	2.2×10^{5ab}	2.5×10^{4b}	0.0	3.5×10^{4b}
Site III	3.4×10^{5ab}	7.0×10^{4a}	1.0×10^{4}	5.0×10^{4a}
SEM	6.5×10^{4}	1.8×10^{4}	1.1×10^{4}	2.0×10^{4}

[abc]Means bearing different superscripts within the
Same row differ significantly ($p<0.05$) .APC =Aerobic plate counts.SC=Staphylococcal counts.CC=Coliforms counts. FC=Fungal counts. Site I= Sabon-gari. Site II= Zaria city. Site III= Samaru areas. SEM=Standard Error of the mean.cfu/g= Colony forming unit per gram

Microorganisms' counts obtained during the bacteriological survey of the products from the control and the various production sites showed relatively high APC in the samples. These counts were however within the upper limit set by (18) in which mean APC of sliced meat products should not exceed 10^6cfu/g. According to (19), influence of environmental sanitation on the microbial population is a highly significant factor in the quality of *Suya* and *Kilishi*. (4) recorded APC of less than 10^6cfu/g for Balangu and *Kilishi*, (20) also recorded APC 10^5cfu/g for *Kilishi* from various sites in Zaria and (21) recorded an APC acceptable limit of 10^5-10^8cfu/g suggested by (22) for processed ready-to-eat chicken.

There was however an exception for the control *Kilishi* and *Kilishi* from Site II that did not record any counts for Coliforms. *Kilishi* from Sites I and III recorded very high CC of 10^4cfu/g against the maximum standard of 10^2cfu/g.

Coagulase positive *S.aureus* was present in the products from all the sites (Tables 4) except in the control *Kilishi*. This high SC is a point of public health concern since the growth of *Staphylococcus aureus* to a population of 10^5cfu/g is considered necessary for the production of 1µgof enterotoxin sufficient to cause intoxication if such food is consumed. However, (18) indicated that 10^6 cells of *S.aureus*/gram are required to present the risk of intoxication. The presence of

Table 4: Biochemical characterisation of the bacterial isolates from *Kilishi* samples

Trt	gmrxn/CM	Catalase	Coagulase	Indole	Citrate	Motility	TSI	MR	VP	Organism
Control	-	-	-	-	-	-	-	-	-	None
$S\text{-}I_1$	+vecocci in clusters	•	+	-	-	-	-	-	-	*S.aureus*
$S\text{-}I_2$	+vecocci in clusters.	-	+	-	-	-	-	-	-	*S.aureus*
	-ve rods(short)	-	-	+	-	+	A/AG	+		*E.coli*
$S\text{-}II_1$	+vecocci in clusters	-	+	-	-	-	-	-	-	*S.aureus*
$S\text{-}II_2$	+vecooci	-	+	-	-	-	-	-	-	*S.aureus*
$S\text{-}III_1$	-ve short rods	-	-	+	-	+	A/AG	+	-	*S.aureus*
	+vecocci in clusters	-	+	-	-	-	-	-	-	
$S\text{-}III_2$	+ve cocci in clusters	-	+	-	-	-	-	-	-	*S.aureus*

Keywords: gmrxn/CM = Gram reaction per cell morphology. MR = Methyl red. VP= Vogesproskauer . TSI = Triple sugar iron. += Positive reaction. - = Negative reaction. S. aureus = Staphylococcusaureus. E. coli= Escherichia coli. Trt: treatment; S -$I_{1\&2}$: Site $I_{1\&2}$ (Sabon-gari);$S\text{-}II_{1\&2}$: Site $II_{1\&2}$ (Zaria city); $S\text{-}III_{1\&2}$: Site $III_{1\&2}$ (Samaru area)

high levels of this coagulase positive *Staphylococci* indicated man and environmental contamination (23) and this is in agreement with earlier reports of(24).

Also, the isolation of Coliforms in some of the products could be an indication of contamination and re-contamination, a theory described by (25). *E. coli, Klebsiella sp* and other Coliforms are organisms of intestinal origin which get into foods through indiscriminate touching by handlers and buyers with poor sanitary habits (4). The fungal species isolated from the *Kilishi* products also pose high health risks to consumers. Growth of moulds on meat can cause spoilage of the affected parts and they can also release toxins into food. If consumed in food, they can, in a long term, have carcinogenic effects. A number of researchers (26, 21, 27, 28) have also reported same trends of findings whereby *Kilishi* and *Suya* products sold in Nigeria are contaminated with various species of bacteria and fungi. (29) stated that the source of contamination on these products could also come from the utensils, from the air and from the spice ingredients because according to (30), spices may serve as a source of contamination to processed meat products.

Conclusion and Application

The results of this study showed acceptable levels of nutritional composition of the *Kilishi* products from the different production sites and the control. However, the microbial loads observed were higher in the commercial samples than in the control products and this level of contamination is at the point of being potentially hazardous to consumers.

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