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ORIGINAL RESEARCH ARTICLE

Processing Characteristics and Micro-structural Evaluation of *Kilishi* under Different Processing Methods

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ABSTRACT

Kilishi processing is time consuming, rigorous and location specific – the focus of this study was therefore to assess if the effects of using a mechanical slicer (rather than hand slicing) and oven drying (instead of sun drying) would significantly affect the product yield of Kilishi. Two kilogram each of choice trimmed beef was used in two replicates of four treatments in a completely randomized design. Treatments A, B, C, and D represents hand sliced, sun dried samples; mechanically sliced, sun dried samples; hand sliced, oven dried samples and mechanically sliced oven dried samples respectively. Meat used were chilled overnight and processed the following morning. The histological characteristics of muscle tissue used for Kilishi production as affected by drying were studied. For slice thickness, there were no significant differences between the mechanically sliced and hand sliced, sun dried products, but the oven dried products were significantly different (P<0.05). It was observed that the mechanical slicing and oven drying gave the highest yield of 65.21 %, compared to 58.66% yield from the traditional processing method. Light microscopy showed that both intracellular and extra cellular components were affected by the conditions of processing. Mechanical slicing and oven drying in Kilishi production holds an economic advantage to producers who desire higher product yield and a safer product from oven drying for consumers who relish the snack.

Keywords: *Kilishi,* processing characteristics, slicing, drying, microstructure,

INTRODUCTION

Kilishi, a tropical intermediate meat type is made from beef slices infused in slurry of defatted groundnut paste and spices and sun-dried. Its ability to keep for several months at room temperature is fast making it a household name, a rich and nourishing snack and a source of supplementary animal protein formulated using hurdle technology, a concept described by Leistner (1987). Salt, sodium nitrite, dehydration or sun-drying and packaging are hurdles applied in sequence to inhibit deteriorating microorganisms (Biscontini *et al.,* 1996) or possibly selecting for desirable flora. Offer *et al.* (1989) gave an extensive review of the structure of meat, and its relationship to water holding capacity, texture and colour. Torres *et al.* (1994), observed that biochemical and physiochemical changes occur during the processing of Charqui (an intermediate moisture meat) and used such parameters to determine product quality. In the same vein Biscontini *et al.* (1996) observed microscopic changes in structure of a meat product (Charqui) exposed to a high salt concentration, followed by solar dehydration.

According to Igene *et al.* (1990), the yield of the product in *Kilishi* production is calculated as apparent or actual percentage yield. Apparent yield was expressed as the ratio of the final weight of the product to the initial weight of the fresh meat whilst the actual yield was expressed as the ratio of the final weight to the fresh sliced meat sheets. A product yield of 54% was reported by Igene *et al.* (1988a) in the traditional production of a 4 kg choice lean beef slices (average 0.2 cm thick, 140 cm long). However, recent studies designed to improve product yield resulted in 75-87% increased yield (Igene *et al.,* 1993) from a combination of improved unit operations, ingredient processing and formulation and use of additives when compared with traditional process, which yields only 54 percent. Much research has been done on the processing characteristics and drying behaviors of *Kilishi* but, there is little on the histology and other properties of this dried meat product. The present study was therefore evaluated to study the structural changes and processing characteristics of *Kilishi* under different processing methods.

MATERIALS AND METHODS

Meat selection, processing and processing characteristics

Two kilogram each of choice trimmed beef from the Semimembranosus was used in two replicates of four treatments (A, B, C and D) in a completely randomized design. Fresh meat samples were chilled overnight at – 4 to 0 °C and processed the following morning according to the method of Ogunsola, (2006).

The treatments were:

A Hand sliced, sun dried samples
B Mechanically sliced, sun dried samples
C Hand sliced, oven dried samples
D Mechanically sliced, oven dried samples

A fabricated mechanical device was used to slice meat allotted to treatments B and D, while the '*Kilishi* knife' was used to manually slice treatments A and C. The meat pieces were cut into sizable, easy-to handle pieces prior to chilling. The slices were set to give a size thickness range of 0.16 to 0.26mm. The drying of *Kilishi* was a two-stage process; the thin slices were first sun dried at the prevailing temperature of 36-40 °C for 6 hours on silver trays. Treatments C and D trays were put in the oven to dry at 50 °C to 60 °C for 4 hours, while treatments A and B samples were sun dried for 6; hours at a temperature of 36 °C to 40 °C. After soaking in slurry of defatted groundnut paste and spices for one hour, the second stage of sun drying under similar conditions to those of the first stage was carried out following the procedure of Igene *et al.* (1990).

Measurements

Slice thickness was measured using Vernier calipers (Caliper gauge polished steel B71/0162) for the dried meat and the final product after the second stage drying and roasting. Other parameters measured include; weight of fresh (raw) meat, chilled weights, weight after first drying, wet infused weight, dried infused weight, and final product weight (after heat sealing). These parameters were used to calculate the product yield, rate of moisture loss and the slice thickness of the raw dried meat slices and the final product; the formulae used for calculations are given below. Parameters calculated from data generated include product yield (for dried meat and final product). The rate of moisture loss was measured in kg/hr before infusion (that is for the dried meat pieces) and after infusion for the (almost finished *Kilishi* product) and calculated using the formulae;

$$\text{Rate of moisture Loss (kg/hr)} = \frac{\text{Wet weight} - \text{dried weight}}{\text{Time of drying}}$$

Chilling loss and the mean slice thickness before and after processing were also determined

$$\text{Dried meat sheets (\%)} = \frac{\text{Dried weight} \times 100}{\text{Sliced fresh weight}}$$

$$\text{Chilling loss (\%)} = \frac{(\text{Warm weight} - \text{chilled weight}) \times 100}{\text{Warm weight}}$$

$$\text{Final product yield} = \frac{\text{Weight after roasting} \times 100}{\text{Wet infused weight}}$$

$$\text{Product yield (\%)} = \frac{\text{Initial weight} - \text{Product weight}}{\text{Initial weight}} \times 100$$

Microstructure of raw meat and *Kilishi*

The *Semimembranosus* muscle was used for the microstructural studies to assess structural changes in raw muscles and the final *Kilishi* product. Samples were prepared from beef of freshly slaughtered 4 year old bull from the slaughter slab of Animal Science, University of Ibadan. Samples used were from three different steps during product preparation – these were raw meat (control), dried meat and the infused slightly roasted sun dried meat, which is the final product. Sliced samples of about 20mm length and 4mm diameter were randomly taken to give representative samples of the raw, dried, infused dried and slightly roasted (the final *Kilishi* samples).

Each sample was soaked in 10% formaldehyde solution for 18-24 hrs. After fixation, the tissue was dehydrated in graded levels of alcohol (ethanol) ranging from 70-100% at intervals of 1hour each for 5-6 hours. Clearing was sequel to dehydration and it involved the removal of alcohol (Ethanol) that the tissues had bathed in by xylene in order to initiate and complete a process that would make cells transparent at microscopic level. It prepared the tissues for infiltration in molten paraffin wax. This step was followed by pressuring and infiltrations, which involved clearing the tissue then embedding in paraffin wax in a wax oven at a temperature of 56-58 °C for 6 hours to penetrate the intracellular spaces of tissue with paraffin wax thus making them more amenable to sectioning. Processed, infiltrated tissues were positioned in blocks in disposable polystyrene plastic trays with paraffin wax for embedding/blocking. The molten wax was first separated from debris by decanting them into the polystyrene plastic trays containing the tissue and labeled accordingly. The blocked tissue was then sectioned with the jung rotary microtome at 5 micron (u) thickness. The sectioned tissue was recovered from the wax by introducing the sectioned tissue into a water bath at 40-50 °C. The sectioned tissue was picked up by a defatted slide.

The tissue was stained using H & E (Haematoxylene and Eosin stain (a routine staining method) and covered with a slip. Staining procedure involved the following: Sections were dewaxed in xylene thrice for 5 minutes each, then cleared in a series of alcohol namely: Absolute (2 changes) at 3-4 times each; 90% (3 minutes) and 70% (2 minutes), then rinsed in water. These were

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stained with Harris Haematoxylene for 15 minutes and rinsed in tap water. The stained tissues were differentiated in acid alcohol for 5 seconds, and then rinsed in tap water for 15 minutes to remove excess dye. It was then treated with 70% alcohol for 3 minutes, counter stained with eosin for 5 minutes to give background colour. The repeated dehydration sequence of 70% (2 minutes) and 90% (2 minutes) were carried out. Absolute alcohol (2 changes per 3 minutes each), xylene (3 changes for 3 minutes each) was then followed. Stained, well-dehydrated sections were then mounted gently with cover slips using "DPX" mountant, and slides allowed to dry before further examination. While mounting sections were kept moist with xylene. Slides were examined under the microscope for uniform staining before mounting. The prepared permanent slides were identified, selected and viewed under Nikkon phase contrast microscope for gross structural appearance. The slides were viewed at x 125, x 250 and x 400.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using SPSS (1983). Significant differences between means were separated using Duncan's multiple range test.

RESULTS

Yield of Kilishi

Data presented in Table 1 shows that there were significant differences ($P < 0.05$) observed amongst all the treatments as regards chilling losses. The trend observed in the chilling losses naturally affected the yield of dried meat slices. The hand sliced, sun dried *Kilishi* (A) and the mechanically sliced sun dried *Kilishi* (B) significantly differed from the hand sliced oven dried (C) and the mechanically sliced oven dried (D) *Kilishi* samples. Treatments A and B did not differ significantly from one another neither did C and D differ from one another with reference to the yield of dried meat slices. No significant difference ($P > 0.05$) was observed in the yield of the final product percent for treatments A, B, and C while treatment D with the highest yield of 65.21 ± 0.77% differed significantly from all the other treatments.

The rate of moisture loss (measured in kg/hr) for the dried meat pieces before infusion and after infusion for the product showed that the meat slices in treatment D had the least moisture loss before infusion (0.18 ± 0.01kg/hr) and after infusion (0.26 +0.01kg/hr). Treatments B and C did not differ significantly ($P>0.05$) in their rates of moisture loss before infusion, both however had lower losses than treatment, A which experienced the highest moisture loss before infusion. After soaking the dried meat pieces in the slurry for one hour and drying, the moisture losses recorded for treatments B and C were similar with just a slight difference from that of A. For slice thickness of the dried meat slices, the hand sliced sun dried (A) and oven dried (C) were comparable. They gave the thinnest slices 0.16 ±0.01cm in comparison to that of treatments B, and C which had the highest slice thickness of 0.26 ±0.01cm. In the final product, slice thickness varied from treatments A to D to C and B each significantly different ($P<0.05$) from one another. The mechanically sliced sun dried treatment B gave the highest slice thickness of 0.43 ± 0.01 while the least (0.32 ± 0.01) cm was obtainable in the hand sliced sun dried treatment A.

Table 1: Product yield and processing characteristics of *Kilishi* as affected by rate of moisture loss, slice thickness, and drying time

Processing characteristics	A	B	C	D
Chilling loss (%)	5.00 ± 0.00^{c}	5.00 ± 0.00^{c}	3.00 ± 0.00^{b}	1.50 ± 0.00^{a}
Yield (dried meat slices%)	35.38 ± 1.53^{a}	40.34 ± 1.76^{ab}	42.78 ± 1.54^{bc}	46.50 ± 0.19^{c}
Yield of final product (%)	58.66 ± 0.90^{a}	57.51 ± 1.95^{a}	56.63 ± 0.92^{a}	65.21 ± 0.77^{b}
%Increase in product yield	23.28 ± 0.10^{c}	17.17 ± 0.11^{b}	13.85 ± 0.01^{a}	18.71 ± 0.12^{b}
Rate of moisture loss (kg/hr)				
Before infusion	0.21 ± 0.01^{c}	0.19 ± 0.01^{ab}	0.18 ± 0.01^{ab}	0.17 ± 0.01^{a}
After infusion	0.28 ± 0.01^{ab}	0.31 ± 0.01^{b}	0.30 ± 0.01^{b}	0.26 ± 0.01^{a}
Slice thickness				
Dried meat slices (cm)	0.16 ± 0.01^{a}	0.26 ± 0.01^{c}	0.16 ± 0.01^{a}	0.19 ± 0.01^{b}
Final product (cm)	0.32 ± 0.01^{a}	0.43 ± 0.01^{d}	0.39 ± 0.01^{c}	0.35 ± 0.01^{b}
% Increase	100.00	65.38	143.75	84.21

^{a,b,c} Means with different superscript in the same row are significantly different ($P<0.05$)

A = Hand slicing + sun drying B = Mechanical slicing + sun drying

C = Hand slicing + oven drying D = Mechanical slicing + oven drying

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Micro structural Studies

The histological structure of raw, dried *Semimembranosus* and that of the Kilishi (dried infused + finished product) are presented in plates 1 to 3. The photomicrographs were taken at x 125 and x 250, for the bovine *Semimenbranosus* (Plates 1-3).

Plate 1: Photomicrograph of the transverse section of raw bovine *Semimembranosus* muscle at x 250

Muscle bundles were intact, with little or no muscle fibre disintegration. The nuclei were slightly seen arranged peripherally as dark specks on the muscle fibres. This was more pronounced for the bovine plates at x 250. The perimysial loose connective tissues containing the intermuscular fat are more pronounced in the muscle fibres.

Plate 2: Photomicrograph of dried bovine *Semimembranosus*

The photomicrograph of the air-dried bovine Semimembranosus muscle (plate 2) reveals that the muscle fibre bundles have shriveled in size. There were clusters of muscle fibre bundles loosely held together intercepted by what looked like breaks or partial cracks across the muscle bundles. The dehydration process appeared to have created perimysial voids along with these cracks which are more visible. There are more muscle fibre bundle units per unit space than it was in the fresh muscle.

Plate 3: Photomicrograph of infused air dried beef Kilishi

The air dried bovine meat pieces rehydrated by infusing in slurry and then dried again have the photomicrograph shown in plate 3. There were more fiber cells per unit bundle and generally more unit cells per unit space. There appeared to be less cracks visible following infusion. The connective tissue of the perimysium here had absorbed fluid and materials from the infusion which caused it to swell filling closely the spaces between the fibres. On subsequent drying, perimysial void are still visible but to a reduced extent.

DISCUSSION

Processing characteristics

In the meat curing industry product yield is considered a very important factor. Several factors affect yield. These include the addition of phosphates-primarily used to reduce excessive shrinkage or 'purge' (cook out) and to increase water-holding capacity of the available proteins, without increasing the apparent saltiness of the product. Other factors that affect yield are smoking, processing time, humidity and the packaging medium used. The proteineous materials such as hydrolyzed plant protein; vegetable protein and monosodium glutamate are also known to help in binding water within the product (Ogunsola, 2006). In the present study, nothing was used to enhance the yield. The traditional processing was followed except where the mechanical slicer was used or oven drying was applied. As noted in the results, only treatment A (hand sliced, sun dried) which is the traditional preparation was significantly ($P<0.05$) lower than that of treatment D (mechanically sliced, oven-dried) in the yield of dried meat slices (Table 1). It has been found that both sodium chloride and sodium tri polyphosphate (STPP) affect textural qualities such as cohesiveness, chewiness and fibre swelling (Offer *et al*, 1983; Young and Lyon, 1997; Young *et al.*, 2005) in addition to increasing cook yield in comminuted product (Lesiak *et al.*, 1995). Igene *et al.* (1993) reported that 0.3% STPP when added to the slurry in *Kilishi*, resulted in a higher actual yield and apparent yield of 77.27% and 72.43% respectively over the traditional process technology. In this study, the actual yield obtained for the traditional processing was 58.66% while the mechanically sliced and oven dried *kilishi* gave a higher yield of 65.21%. The latter was higher than what was obtained by other workers (Igene, 1988a, Igene *et al.*, 1990) who obtained a yield of 54% for the traditional processing. Otherwise, there was no significant difference between the traditional processing (Treatment A) and Treatments B and C. The percentage increase in product yield which is the increase in yield of the final product above that of the corresponding dried meat slices for the traditional processing was highest and differed significantly from those of other treatments, while that for Treatment D was the least.

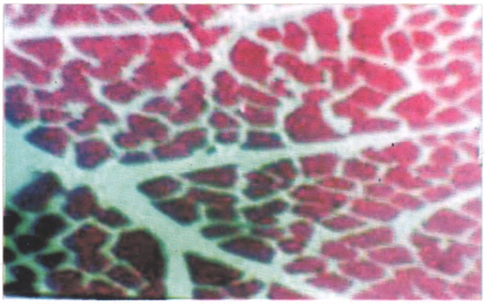

Plate 1: Photomicrograph of raw bovine semimembranosus muscle (x 125)

Rustard and Nesse (1983) reported that the functional properties of foods are affected by higher moisture contents since moisture serves as a vehicle for transport of substance within the food matrix (Lonnauro *et al.*, 1985). According to Igene (1990), a moisture content of

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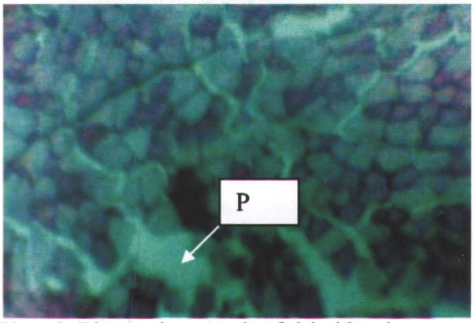

Plate 2: Photomicrograph of dried bovine semimembranosus (x 125)

(P shows the connective tissue of the perimissium with perimysial voids)

40-50% is the best moisture level for terminating the first stage of drying during *Kilishi* processing. In this study however, the moisture content at the first stage drying was lesser (35-46%). Yield depends to a large extent on the ability of meat proteins to open up and infuse the ingredients. It would seem plausible to suppose that optimum moisture content exists within and outside the moisture percentage of 40-50% at which not much protein is damaged as to hamper ingredients infusion and retention at the same time keeping the quality attributes of the product (Igene *et al.,* 1993). The rate of moisture loss during the first stage of drying before infusion for the hand sliced, sun dried *Kilishi* was higher than that of the other treatments; while the mechanically sliced, oven dried *Kilishi* lost the least moisture. After infusion however a slightly different trend was observed. This parameter is important because in processing the faster the rate of drying the less likely it is that contaminants would come in contact with the product, thus ensuring product stability and extending the shelf life. In *Kilishi* processing, slicing is used rather than cutting the meat in chunks in order to allow for proper and quick drying of meat slices so as not to predispose the meat to microbial attacks while processing.

Thin slicing has been reported to enhance air drying of products with recommended slicing of 0.17-0.31cm for *Kilishi* (Igene *et al.,* 1990), 0.05- 1.5cm for Okra (Adom *et al.,*1997) and for lean bacon (Konstance and Pancer, 1985). In Kilishi and bacon drying, it was reported that their drying comes in two stages. Sliced samples before infusion exhibited a constant drying rate, followed by a falling rate period with adjoining critical moisture content of 1.75kg H_2O / kg solid, but a constant rate of drying was not observed during the second stage drying after infusion (Igene *et al.*, 1993). In this study the least slice thickness was obtained in the hand sliced, sun dried *Kilishi* both before and after infusion, this was followed by the mechanically sliced, and oven dried Kilishi (Table 1). The study has shown that treatments B and C may not really be recommended for *Kilishi* production because of the lower yield they offered, their higher moisture loss rate and the higher slice thickness obtained. The mechanically sliced oven dried Kilishi gave the highest yield and is therefore recommended.

Micro structural studies

Skeletal muscles have a wide variety of morphological forms and modes of action, nevertheless all have the same basic structure being composed of extremely elongated multinucleate cells, often described as muscle fibres, and bound together by connective tissue. Individual muscle fibres range considerably in diameter from 10-100 um and may extend throughout the whole length of a muscle reaching up to 35 cm in length (Wheater *et al.,* 1994). The bovine Semimembranosus muscle shows the muscle fibres, both primary and secondary bundles with the associated endomysial and perimysial connective tissues (Plate 1). While histological traits could sufficiently predict raw meat tenderness (Okubanjo, 1978a, 1978b) it might not be so with cooked meat (Chambers *et al.,* 1982). There was a fairly even distributed thin-layered perimysial and endomysial connective tissues.

Traditionally, the freshly excised muscle is used in *Kilishi* preparation but it was found out that slicing the raw meat with the mechanical slicer was an impossible task. The meat therefore had to be chilled to make the mechanical slicing achievable. The photomicrograph of the air-dried bovine *Semimembranosus* muscle revealed that the muscle fibres had shriveled in size (Plate 2). Fresh meat contains about 70% water by weight and keeps the fresh muscle turgid.

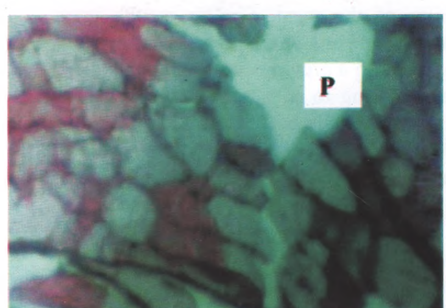

Plate 3: Photomicrograph of kilishi made from bovine semimembranosus (x 250)

(P shows the connective tissue of the perimissium with perimysial voids)

Removal of appreciable water during dehydration allows the cells to collapse causing the shriveling observed in the dried meat slices. The dehydration process appears to have given space to perimysial and endomysial cells to be more visible. There are more muscle fibre units per unit space than it was in the fresh muscle. According to Hedrick *et al.,* (1994) meat products dried by hot air shrivel considerably, and have poor rehydration properties due to the protein denaturation that occurs during the drying operation. Hot air drying predisposes pork fat to rancidity, which was prevented by appropriate packaging to eliminate oxygen in the study, though antioxidants could also be added. Dehydration of bovine *Semimembranosus* muscle and indeed any other muscle results in physical disruption of both inter and intra cellular structures (Plates 2).

Physical manifestation of this disruption can be readily seen in the extensive cracks in the perimysium. The cracks result in the formation of perimysial voids as perimysial protein collagen is denatured and becomes more friable. The individual muscle fibre also loses water as the cell wall loses its ability to regulate inward and outward movement of water. When the dried meat slices are soaked in the infusion slurry, extensive uptake of fluid and materials by the cell become manifest. The denatured meat collagen absorbs water swelling greatly while the infusion also migrates inward into the interior of the muscle fibres which also get distended thus; all the perimysial voids were filled up. In subsequent drying and slight roasting after infusion, less perimysial cracks and voids were observable as most of them had been filled up with the infused materials as well as the surface of the meat slices. The final product is usually heat sealed by slightly roasting on fire. Palka (2003) observed that roasting bovine *Semitendinosus* muscle to an internal temperature of 50 °C slightly affected the structure of the meat. Similar results were obtained during the retorting of the bovine ST muscle to the same temperature in a previous study (Palka and Daun, 1999). During roasting to an internal temperature of 60-90 °C significant changes occurred both in myofibrils and in the intramuscular connective tissue. Palka (2003) also observed that the relationship between the texture of raw and roasted meat is rather limited.

CONCLUSION

The result showed that the mechanical sliced and oven dried samples gave the highest yield of 65.21 %, compared to 58.66% yield from the traditional processing method. The slice thickness of the final product varied in all the treatments but the traditional processing still gave the lowest slice thickness. Also, light microscopy result showed that both intracellular and extra cellular components were affected by the conditions of processing. Mechanical slicing and oven drying in *Kilishi* production holds an economic advantage to producers who desire higher product yield and a safer product from oven drying for consumers who relish the snack

CONFLICT OF INTEREST

I certify that this is an original research work carried out by me as part of my doctoral thesis, which has not been published elsewhere prior to this submission.

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