



Assessment of Products of Lipid Oxidative Chemical Changes in *Kilishi* Prepared from Fermented Cattle and Camel Beef in Semi-arid Nigeria

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Abstract

A shelf-life study was conducted at the Department of Animal Science, Bayero University Kano and the products of lipid oxidative chemical changes in fermented cattle and camel beef Kilishi examined. The effects of meat type, age and packaging on this important meat quality attribute was evaluated. The experiment, which lasted for six months, was a 2 x 3 x 4 factorial arrangement in a completely randomized design. Triplicate samples were collected and data generated analysed by analysis of variance using SPSS Version 20.0 and GraphPad Instat and significantly different means separated with Tukey HSD test. Meat type and age had no significant ($P > 0.05$) effect on lipid chemical changes. The peroxide (PV, 1.06 meq/kg), acid (AV, 4.82 mg/g), saponification number (SV, 146.02 mg) and iodine (IV, 61.17 g) values were significantly ($P < 0.05$) higher in brown paper+PVC, brown paper and PVC packages, respectively. Significant ($P < 0.05$) interaction effect was observed in acid value and refractive index with respect to age, meat type and package. The results of the analysis of lipid chemical changes in the stored Kilishi indicated that the values were either below or above the limits specified for dry meat products. Kilishi can thus be stored for an extended period of time under ambient temperature when properly packaged.

Keywords: shelf-life, meat type, packaging, meat quality

Introduction

The increase in demand for livestock products represents a major opportunity for livestock producers to expand their businesses by expanding their livestock stock and investing in productivity-enhancing technologies. In 2018, an estimated 69 billion chickens; 1.5 billion pigs; 656 million turkeys; 574 million sheep; 479 million goats; and 302 million cattle were slaughtered for meat production (Hannah & Max, 2020). Maintenance of product quality and improvement of shelf-life stability is of paramount importance in meeting an unprecedented increase in demand.

Lipid oxidation is a major cause of meat quality deterioration and an important determinant of shelf life of meat and meat products (Sudip *et al.*, 2014). Reactions of oxidative characters occurring in meat and meat products are the major causes of deterioration in their quality especially with respect to colour, flavour, texture and slime formation, as well as nutritive value

(Casaburi *et al.*, 2015). Lipid oxidation leads to the formation of free radicals and hydroperoxides (Akamittath *et al.*, 1990). Hydroperoxides are not stable and decompose rapidly to secondary oxidation products such as aldehydes and ketones (Nawar, 1996). In assessing the various stages of oxidative changes, iodine value (IV), saponification value (SV), refractive index value (RV), peroxide value (PV) and thiobarbituric acid reactive substances test (TBARS) are determined to identify and measure primary and secondary oxidation compounds. All these quality parameters, except iodine and refractive index values, increased during storage (Flavia *et al.*, 2014).

Peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation (El-Shater *et al.*, 2012). Peroxide value can be used to measure primary and secondary oxidation products and thus infer the extent of oxidation (Dave

& Ghaly, 2011). These secondary products can cause loss of colour and nutritive value due to severe effects on lipids, pigments, proteins, carbohydrates and vitamins (Simitzis & Deligeorgis, 2010). Acid value test measures free fatty acids present in a given amount of lipid as an indication of hydrolytic rancidity (El-Shater *et al.*, 2012). The pH-value or acidity of meat is important in relation to its microbiological and keeping quality. Acid value in fat should not be more than 0.6 - 0.8 mg KOH/g (NRA, 2008).

Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali. The lower the saponification number or value, the larger the molecular weight of fatty acids in the glycerides present and vice versa. The Iodine Absorption Value is an indication of the degree of unsaturation in lipids. The iodine value (IV) or "iodine absorption value" or "iodine number" or "iodine index" is the mass of iodine in grams that is consumed by 100 grams of a chemical substance (Mustapha, 2016). Since unsaturated fatty acids are 'softer' or less firm, iodine value can be used as indicator of overall carcass fat firmness (Benz, 2008). Research has indicated that the maximum acceptable iodine value should be 70 to 75 g per 100 g (NRA, 2008). Refractive index is a relative value indicating the refraction of light rays moving through thin layers of fat. It is related to the degree of binding saturation, but it is affected by other factors such as free fatty acids content, oxidation and thermal treatment (Muniz *et al.*, 2015). It is possible to determine the salt, protein, fat and water content of a piece of meat by the measurement of its density (Torres *et al.*, 2010)

The objective of this study was to monitor the rates of lipid oxidative changes in fermented cattle and camel beef Kilishi as a storage quality control measure.

Materials and Methods

The experiment was conducted at the Teaching and Research Laboratory of the Department of Animal Science, Bayero University Kano. Kano State is located in the semi-arid area of north western Nigeria. Kano has a hot semi-arid steppe climate which exhibits a tropical wet dry season with a mono modal rainfall distribution. Rains typically start in May with a few shower, stabilize in July, with highest amount in August and ends in September. The mean annual rainfall in Kano State fluctuates from south to north varying from 600 to 1200 mm and 300 to 600 mm respectively (KNSG, 2004). Ambient temperature varies sharply depending on the season, ranging between 19.6^oC in December to January to 40^oC in March to April (Olofin, 1987). The mean monthly sunshine hours vary from 220.1 to 266.6 (KNSG, 2004). Air humidity is high during the wet season and very low during the dry season. It ranges between 17 to 64% for the average daily low and high levels (Weatherspark, 2013).

Meat animals, both large (cattle and camels) and small (sheep and goats) are slaughtered daily at various locations in the State including the Kano Main Abattoir. These animals are sourced from local, regional and international markets in Nigeria and neighbouring countries. The livestock slaughter rate reflects the human population of the State as large number of large and small ruminants and poultry are slaughtered on daily basis and the meat either sold fresh or processed into various ready-to-eat snack products such as *Tsire*, *Balangu*, *Dambun Nama* and *Kilishi*.

Meat Handling and Processing for Fermented Kilishi Production

After deboning, removal of visible fat and connective tissues, the chunks of meat were sliced and then inoculated with Lyocarni' (SACCO Sri Cadorago Co., Italy) dried lactic meat starter culture. Meat samples from the semi membranous muscles of cattle

and camel were smeared gently and thoroughly with the solution. The meat was inoculated in 100 ml/kg and incubated for 4 hrs at 40°C (Olaoye & Ntuen, 2011) while the fresh untreated beef samples were used as control. For the purpose of the second experiment, raw cattle and camel beef (30 kg each) was obtained and treated as above. The meat samples were obtained from cattle and camels aged 3, 5 and 8 years and above. Animal aging was done by dentition according to the procedure described by Ensminger (1992) and Ferguson (1990). The sliced raw beef samples were then inoculated at 2.5% level of inclusion of LAB meat starter culture as this was found adequate for the fermentation process during the first experiment.

Preparation of Fermented Beef Kilishi

The fermented cattle and camel beef were prepared into *Kilishi* according to a

modification of the procedure described by Jones *et al.* (2001) as shown in Figure 1. The meat slices were reduced to a moisture content of 40-50% after a period of 4-5 hours drying. The dried pieces of meat were then soaked in a coating sauce of groundnut cake and condiments (Table 1) for 15 minutes. The soaked meat was removed and spread out again for the second stage drying. The infused meat slices were carefully turned over periodically to avoid sticking to the surface of the drying platform. The drying stages were undertaken under protective cover against dust and flies. After 4 hours of drying, the infused meat slices were roasted for 5 minutes over a glowing smokeless charcoal (80⁰-120°C) and packaged for analysis (first experiment) or kept for a six months storage period (second experiment).

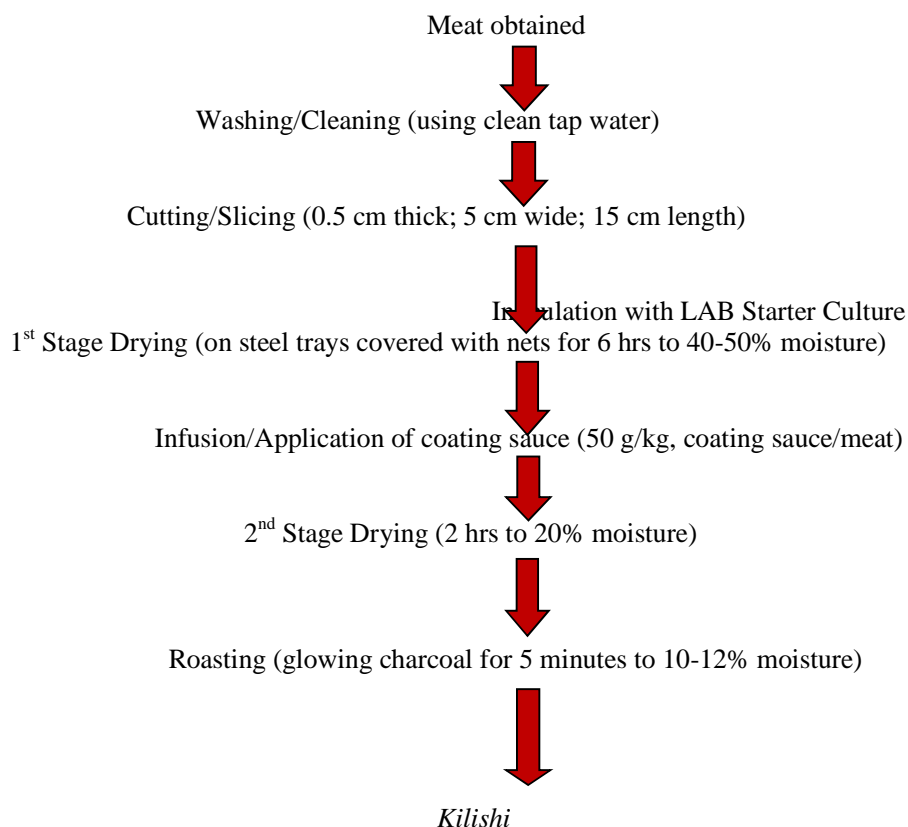


Figure 1. *Kilishi* preparation procedure (Source: Abubakar *et al.*, 2011; Muhammad *et al.*, 2011).

Table 1: Proportions of Ingredients Used in the *Kilishi* Coating Sauce.

Ingredient	Proportion in Mixture (g/Kg)
Black pepper (<i>Piper guineense</i>)	15
Clove (<i>Eugenia caryophyllata</i>)	10
Alligator pepper (<i>Aframomum meleginata</i>)	10
Hot pepper (<i>Capsicum frutescens</i>)	45
Sweet pepper (<i>Capsicum annum</i>)	50
Onion (<i>Allium cepa</i>)	130
Ginger (<i>Zingiber officinale</i>)	25
Garlic (<i>Allium sativa</i>)	15
African nutmeg (<i>Myristica fragrans</i>)	5
Seasonings (Maggi ^R , Thyme ^R , Curry ^R)	30, 10 and 5 (respectively)
Decorticated groundnut (<i>Arachis hypogoeaea</i>)	10
Defatted groundnut cake (<i>Kuli-kuli</i>)	350
Sugar	10
Water	400
Common Salt (Sodium chloride)	30

Source: Mgbemere *et al.* (2011); Muhammad *et al.* (2011); Olusola *et al.* (2012).

Experimental Lay-out, Sampling Procedure and Analyses

The experiment was laid in a 2 x 3 x 4 factorial arrangement in a completely randomized design. The factors are two animal species (cattle and camel) aged 2 to 3, 5 to 6 and 8 years and above and four packaging arrangements: brown paper (B), polyvinyl chloride (PVC), aluminium foil and polyvinyl chloride (AP), brown paper and polyvinyl chloride (BP).

Sample Collection and Analyses

The experimental samples were kept for six months under ambient temperature. Triplicates samples from the stored cattle and camel beef *Kilishi* were taken to the Biochemistry Laboratory, Faculty of Science, Bayero University, Kano for the chemical analyses. The products of lipid chemical changes (peroxide value, acid value, saponification number, iodine value, relative density and refractive index) were assessed for shelf life study and quality control. The refractive index was determined using a portable Refractometer (Erma, Tokyo, Japan).

Statistical Analysis

The data obtained were analysed using the means of three replicates of each sample. The data generated from the results of the chemical analysis of the fermented cattle and camel beef *Kilishi* were analysed by analysis of variance (ANOVA) using the IBM SPSS Version 20.0 (2011) and GraphPad InStat (GraphPad InStat[®], Version 3.05, 32 bits for Win 95/NT, GraphPad Software Inc., (2000) Statistical packages.

Ethical Issues

All general programmes and practices to ensure safety and quality control of the fermented cattle and camel beef *Kilishi* as a ready-to-eat meat product was strictly observed. These include good manufacturing, sanitation and handling practices.

Results

Shelf life study was conducted by examining chemical changes in lipid content of the stored *Kilishi* prepared from cattle

and camel beef. The effect of meat type, animal age, use of different packaging materials and storage time was considered. The initial sampling for the study was undertaken after three months and then six months thereafter. The parameters considered were peroxide value (PV), acid value (AV), saponification number value

(SV), iodine value (IV) relative density (RD) and refractive index (RI). The results in Table 2 indicate the effect of meat type on lipid changes in fermented cattle and camel beef *Kilishi*. The values obtained from the analysis did not reveal significant ($P > 0.05$) differences among the parameters evaluated.

Table 2: Effect of Meat Type on Chemical Changes of Lipid in Fermented Cattle and Camel Beef *Kilishi*

Parameter	Meat Type		SEM (\pm)
	Cattle	Camel	
PV (meq/Kg)	0.89	0.93	0.02
AV (mg/g)	3.80	3.78	0.07
SV (mg)	133.00	133.14	0.99
IV (g)	52.23	52.24	0.65
RD	0.92	0.91	0.01
RI	1.29	1.23	0.02

PV=Peroxide Value; AV=Acid Value; SV=Saponification Number Value; IV=Iodine Value; RD= Relative Density; RI=Refractive Index.

The impact of animal age on the chemical changes of lipid in the fermented cattle and camel beef *Kilishi* is presented in Table 3. The result did not indicate significant ($P > 0.05$) differences among the parameters evaluated. The peroxide values in the *Kilishi* prepared from animals aged 5 and those at 8 years and above were the same but numerically higher than in the product made from animals aged 3 years.

Table 3: Effect of Age on Chemical Changes of Lipid in Fermented Cattle and Camel Beef *Kilishi*

Parameter	Slaughter Age (Years)			SEM (\pm)
	3	5	8 and above	
PV (meq/Kg)	0.85	0.94	0.94	0.03
AV (mg/g)	4.04	3.76	3.56	0.08
SV (mg)	131.56	134.68	132.96	1.21
IV (g)	51.89	52.61	52.21	0.79
RD	0.91	0.91	0.92	0.01
RI	1.24	1.23	1.31	0.03

PV=Peroxide Value; AV=Acid Value; SV=Saponification Number Value; IV=Iodine Value; RD= Relative Density; RI=Refractive Index.

The impact of packaging on lipid changes in the fermented cattle and camel beef *Kilishi* is shown in Table 4. The experimental packaging materials significantly ($P < 0.05$) affected the rate of lipid changes in the fermented *Kilishi*. The PV value of samples stored in the double layer packaging arrangement comprising brown paper and PVC (BP) was significantly ($P < 0.05$) higher. The level of PV in the single layer packages (brown paper and PVC) did not

reveal significant differences. The lowest level of PV was observed in *Kilishi* samples kept in aluminium foil and PVC (AP) package. The level of the acid value depicted the same pattern as in the case of PV as samples in BP recorded significantly ($P < 0.05$) higher content. However, *Kilishi* samples stored in the PVC had significantly ($P < 0.05$) higher AV compared with that kept in the brown paper. The lowest content of AV was found in samples kept in

aluminium foil and PVC double layer package. The result of the analysis of the saponification number value revealed that *Kilishi* samples kept in the brown paper single layer package had significantly ($P < 0.05$) higher level. In terms of iodine value, significantly ($P < 0.05$) higher content was found in *Kilishi* samples kept in the PVC single layer package while those stored in

the aluminium foil+PVC packaging arrangement had the lowest amount. The result of the RD and RI analysis did not reveal significant ($P > 0.05$) differences among the treatments. However, samples kept in the BP package had numerically higher value of relative density while those stored in BP and P recorded similar rates of RI.

Table 4: Effect of Packaging on Chemical Changes of Lipid in Fermented Cattle and Camel Beef *Kilishi*

Parameter	Packaging Medium				SEM (\pm)
	AP	BP	B	PT	
PV(meq/K)	0.76 ^c	1.06 ^a	0.89 ^b	0.93 ^b	0.03
AV (mg/g)	2.76 ^d	4.82 ^a	3.29 ^c	4.28 ^b	0.10
SV (mg)	129.53 ^b	130.38 ^b	146.2 ^a	126.34 ^b	1.39
IV (g)	43.70 ^d	57.01 ^b	47.08 ^c	61.17 ^a	0.91
RD	0.91	0.92	0.91	0.91	0.01
RI	1.18	1.33	1.20	1.33	0.03

Means in the same row with similar superscripts are not significantly different at $p < 0.05$. AP=Aluminium foil+PVC; BP=Brown Paper+PVC; B=Brown Paper; P=PVC; PV=Peroxide Value AV=Acid Value SV=Saponification Number Value IV=Iodine Value RD=Relative Density, RI=Refractive Index.

The results of the analysis of the impact of storage duration on the rate of lipid changes in the fermented cattle and camel beef *Kilishi* is presented in Table 5. Generally, the result did not reveal significant ($P > 0.05$) differences among the parameters evaluated. However, samples kept for 6 months had numerically higher amounts of PV, AV, SV and RD. Furthermore, samples stored for 3 months recorded higher IV and RI.

Table 5: Effect of Storage Duration on Chemical Changes of Lipid in Fermented Cattle and Camel Beef *Kilishi*

Parameter	Storage	Duration	SEM (\pm)
	3 Months	6 Months	
PV (meq/Kg)	0.86	0.96	0.02
AV (mg/g)	3.63	3.95	0.07
SV (mg)	132.93	133.20	0.99
IV (g)	52.28	52.20	0.65
RD	0.90	0.92	0.01
RI	1.28	1.25	0.02

PV=Peroxide Value, AV=Acid Value, SV=Saponification Number Value, IV=Iodine Value, RD= Relative Density, RI=Refractive Index

Interaction Effect of Products of Lipid Chemical Changes in the Fermented Cattle and Camel Beef

A significant ($P < 0.05$) interaction was observed between meat type and package with respect to acid value of the *Kilishi* prepared from the fermented cattle and camel beef as shown in Figure 2. All the packaging media exerted influence in

relation to the type of meat used in preparing the fermented *Kilishi*. The AV of the fermented camel beef *Kilishi* stored in aluminium foil+PVC double layer and brown paper packages was higher than that of cattle. However, *Kilishi* prepared from cattle beef and kept in brown paper+PVC and PVC packages had higher acid values.

The acid value (AV) had also shown significant ($P < 0.05$) interaction with respect to animal age and meat type as presented in Figure 3. There was interaction between the *Kilishi* prepared using 3 and 5 year old cattle while such relationship was found between the product made from 5 years and 8 year

and above camels. The AV of *Kilishi* prepared from the 3 and 5 year cattle was higher compared to that of animals aged 8 years and above. Furthermore, the AV of 3 years camel beef *Kilishi* was higher than that of the 5 and 8 years and above ones.

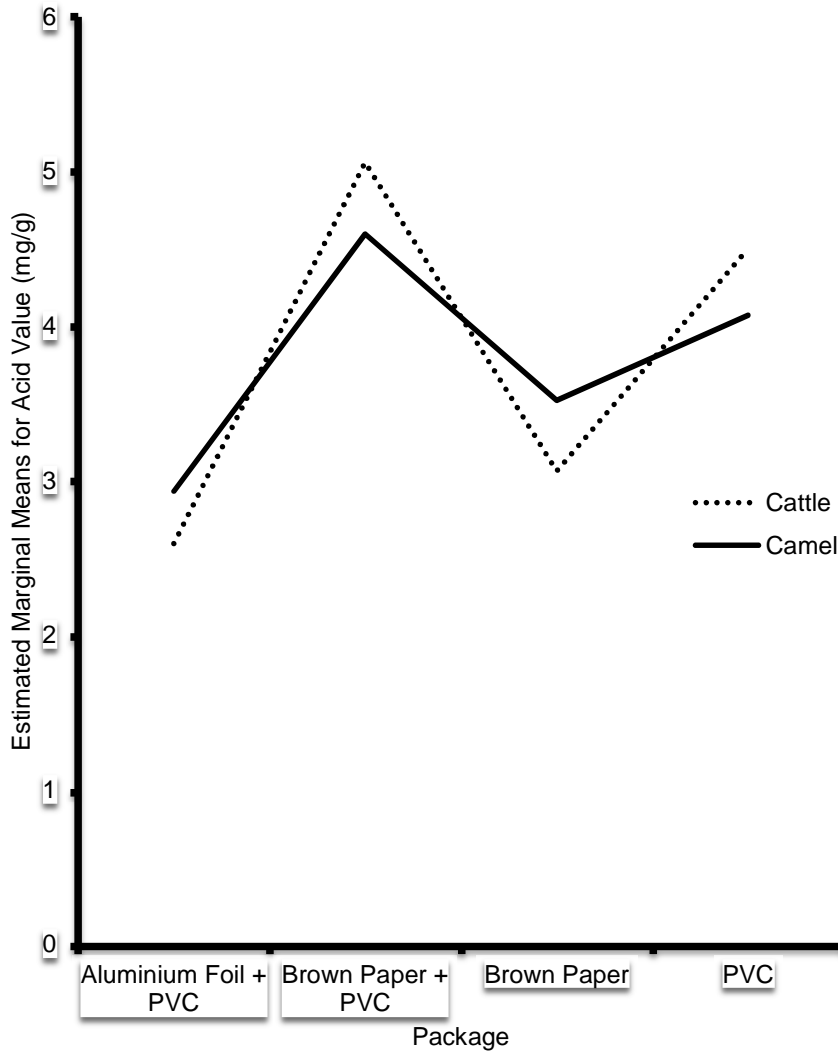


Figure 2. Interaction of Packaging and Meat Type on the Level of Acid Value in Fermented Cattle and Camel Beef *Kilishi*

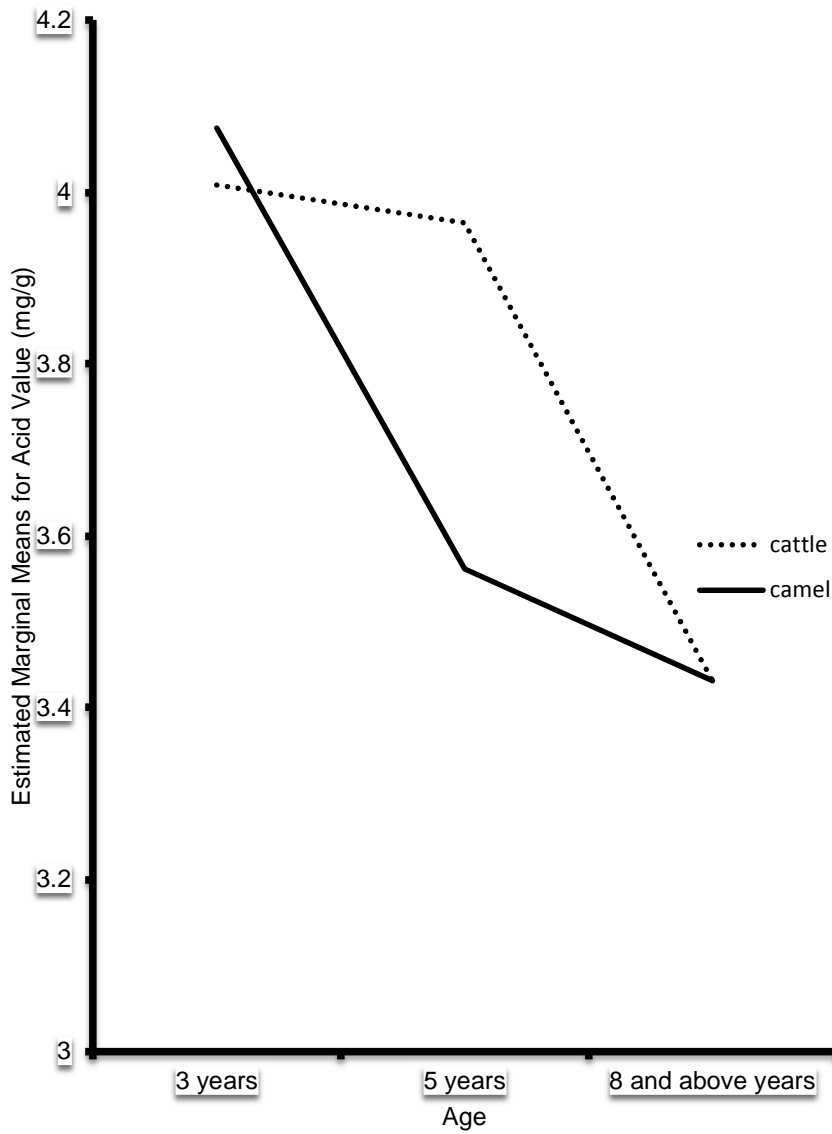


Figure 3. Interaction of Meat Type and Slaughter Age on the Level of Acid Value in Fermented Cattle and Camel Beef *Kilishi*.

The profile plot in Figure 4 showed the interaction of meat type and packaging in relation to refractive index (RI) of the fermented cattle and camel beef *Kilishi*. The RI of the product prepared from cattle beef and kept in aluminium foil+PVC, brown paper and PVC had higher RI values compared to that of camel. However, camel beef *Kilishi* stored in the brown paper+PVC double layer package had higher RI than that of cattle.

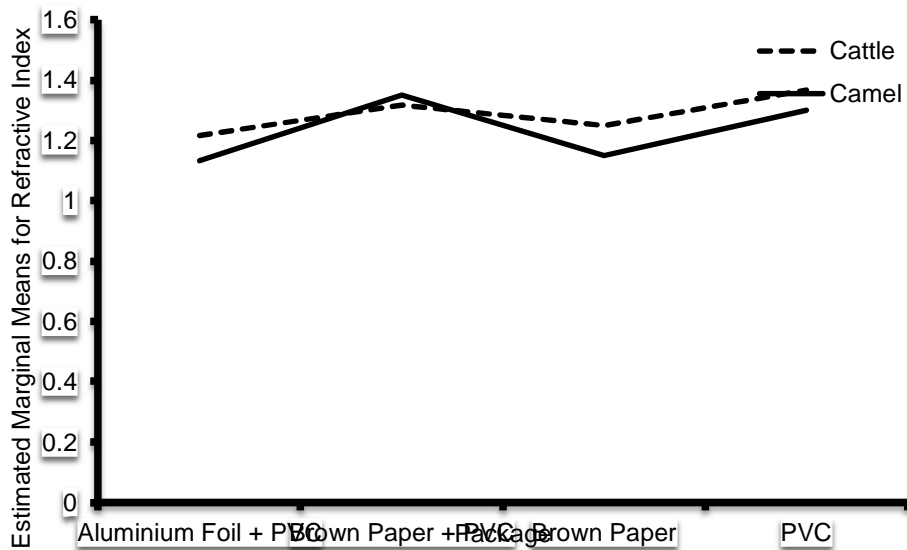


Figure 4. Interaction of Packaging and Meat Type on the Level of Refractive Index in Fermented Cattle and Camel Beef *Kilishi*

Discussion

The rate of formation of products of lipid chemical changes was examined in assessing the shelf stability of the *Kilishi* samples subjected to varying packaging arrangements in storage. The products of lipid chemical changes examined in the current study can exert tremendous impact

on the quality of the fermented *Kilishi* because both the raw meat and the ingredients used in preparing the product contained substantial amounts of fat. However, as result of fermentation of the raw meat and use of efficient packaging materials, the impact of the changes was not pronounced in respect of loss in quality

attributes. The levels of products of lipid oxidative changes recorded after three and six months storage were low even though they exhibited the normal pattern reported in the literature for dry stored meat products (Flavia *et al.*, 2014). There was increase in the amounts of peroxide, acid and saponification number values and the relative density. Slight decreases were observed in levels of iodine value and refractive index. Higher values of peroxides (17.23 – 19.94meq/kg) were reported in Kundi, another semi-dry meat product in Nigeria (Adeyeye, 2016). Igene (1988) reported the lipid and fatty acid composition of *Kilishi* to be stable to lipid autoxidation despite its higher level of unsaturated fatty acids. Also Igene *et al.* (1990) reported that the oxidative stability of *Kilishi* is attributed mainly to the antioxidant properties of the phenolic substances contributed by array of spices and other plant products such as *Capsicum frutescens*, *Piper guineense*, *Capsicum annum*, *Allium cepa*, *Eugenia caryophyllata* and *Aframomium officinale* used in the preparation of the product and concentrated due to low water activity.

Flavia *et al.* (2014) reported a trend of increase in the levels of products of lipid changes during the storage of meat preparations with the exception of iodine and refractive index values which is in agreement with the finding of the current study. The values of most of the parameters examined in this study were either below (PV, SV and RI) or within (AV, IV and RD) the limit values allowed in meat products by FAO (2012) and FHB (2015).

Conclusion

The findings of this study demonstrated that cattle and camel beef could be used to prepare *Kilishi* without much difference in keeping qualities. Fermentation has improved the shelf stability of cattle and camel beef *Kilishi* and the packaging materials protected the products from agents of chemical deterioration. Polyvinyl chloride alone or in combination with other

packaging materials could be used for *Kilishi* as this protect and preserve the product.

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