A Microbiological and Nutritional Evaluation of the West African Dried Meat Product, Kilishi

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Abstract

The quality attributes of Kilishi, a West African dried meat product were studied over an eight week storage period comparing traditional production and packaging systems with a potassium sorbate treatment system and simple modern packaging. Changes in chemical composition and microbiological counts are reported. Moisture and water activity results indicated that the experimental Kilishi was sufficiently dried to minimise microbial growth. Fat oxidation levels measured by free fatty acids (FFA) (%) on extracted fats were unacceptably high (>1.2-2.1%) and may be a reflection of the quality of the groundnut and its oil in the ingredients. Processing of beef into Kilishi appears to lead to a decrease in mineral availability. Results suggest that treatment of Kilishi with 10% (w/v) potassium sorbate confers a degree of protection of the product from mould contamination. Aflatoxin levels far exceeded all established safe limits and are thought to be due to the use of pre-contaminated groundnut, as mould growth levels in Kilishi were very low.

Key-words: **aflatoxins, dried meat, food preservative, kilishi, potassium sorbate.**

Introduction

As it is highly perishable, meat surplus to immediate requirements needs to undergo some form of preservation if it is not to be wasted. Man developed meat preservation technologies from an early period; the history of meat preservation in West Africa dates back to records of the 12th century. The main method of meat preservation transferred by the medieval Arabic sources to West Africa was that of sun drying (Alonge and Hiko, 1981). Although modern methods of meat preservation might be preferred by consumers, refrigeration equipment is expensive to install and difficult to maintain in tropical developing countries, so dried meat products often maintain their importance. There are many methods used to prepare dried meat. These include exposure of strips of lean meat to the sun, as in the manufacture of Pemmican by North American Indians, or a combination of salting followed by air drying, as in the preparation of Charqui (in South American) and Biltong (in South Africa) (Lawrie, 1979). The dried meat product Kilishi is produced mainly by Hausas and Fulanis in the Northern parts of West Africa (Alonge and Hiko, 1981). Kilishi is prepared by partially drying thin sheets of quality beef in the sun followed by marinading in a slurry of ingredients before a second period of sun drying and briefly roasting (Igene *et al.*, 1990; Musonge and Njolai, 1994). There is little or no packaging of the product before presentation to the consumers. This may have an effect on the quality of the final product. This paper reports the findings of a study of the quality of traditional Cameroonian Kilishi and the effect of simple, cheap packaging and treatment with an anti-fungal agent during storage under two different climatic conditions.

Materials and Methods

Kilishi preparation

Traditional Kilishi is prepared using quality beef. It is prepared by skillfully cutting lean meat into thin sheets (1-2mm thick). These sheets of meat are sun dried on a raised wooden table covered in rush matting for about four hours. The sheets of meat are then immersed in a slurry of groundnut caked and seasonings including sugar, salt and paper (Igene, 1988). After immersion, the meat is returned to the rush matting to dry in the sun for a further five to twelve hours. The product is finally roasted briefly over fire. Kilishi production is not standardised and there are many variations of the method described above. Ingredient formulations, infusion time and duration of the solar drying stages vary depending on the required taste and environmental conditions. There are also variations in drying methods and some producers do not employ the final roasting stage.

Trial 1 : **Quality characteristics of traditional Kilishi two days post production**

Representative samples of Kilishi (approximately 2.5 kg) were obtained two days after production from four different producers in Ngaoundere, Cameroon. The samples were weighed and finely ground in a pre-sterilised and chilled waring blender. The ground Kilishi was then subjected to a range of analyses as detailed below. Results are expressed in **Table 2**.

Trial 2: **Quality characteristics of Kilishi during short term storage using potassium sorbate as an anti-fungal agent**

Representative samples of Kilishi (approximately 10 kg) were obtained from a Kilishi producer immediately after production. The samples were subjected to a range of analyses as detailed below after storage under ambient conditions of temperature and relative humidity. Approximately half the samples were stored wrapped in traditional brown paper (control). The other half was heated to 100°C for 10 minutes and then lightly sprayed with 10% (w/v) solution of potassium sorbate in sterilised water, onto the surface of the product before being packaged in polythene bags and stapled (treatment). The heat from the product aided evaporation of the water, leaving the sorbate as an inhibitor. Samples were

prepared for analysis as in trial 1 and then analysed each week for three weeks. Results are expressed in **Table 3**.

Trial 3: **Quality characteristics of Kilishi during long term storage using potassium sorbate as an anti-fungal agent**

Representative samples of Kilishi (approximately 10 kg) were obtained from a producer immediately after production. Approximately one third of samples were stored wrapped in traditional brown paper (control), one third treated with 10% (w/v) potassium sorbate (see trial 2) before being packaged in sealed polythene bags (treatment) 1) and one third packaged in sealed polythene bags (treatment 2) without potassium sorbate treatment. Samples were prepared and subjected to a range of analyses (see below) after 8 weeks storage under ambient conditions. Results are expressed in **Table 4**.

Analysis of Samples

The Association of Official Analytical Chemists methods (AOAC, 1984) were used for the determination of moisture, ash, fat and mineral elements (calcium, magnesium and phosphorus).

Water activity (aw) was determined using a Lufft water activity analyser (Model 5803). A quantity of ground Kilishi was mixed with the same weight of distilled or deionised water and the pH measured with a pH meter (Jenway model 3020). Free fatty acids (FFA) were extracted from Kilishi using the Leatherhead Food R.A. room temperature Bligh and Dyer extraction method No.5 for fat and FFA determined by method No.2 for fat extracted from food (Slack 1987). Protein was determined by the distillation method of Kjeldahl (AOAC, 1984). Calcium (Ca) and magnesium (Mg) were determined by the atomic absorption method (AOAC 1984) and phosphorus (P) was assayed colorimetrically as the phosphomolybdovanadate complex at 400 mm. Aflatoxins were determined by bi-directional HPTLC after extraction in acetone and phenyl bonded-phase cartridge clean-up (Bradburn and Coker, 1995). Aerobic plate count (plate count agar standard, Oxoid CM463), moulds and yeasts (Dichloran Rose-Bengal chloramphenicol (DRBC), Oxoid CM727), xerophilic moulds (Dichloran-Glycerol 18 (DG18), Oxoid CM729) *Clostridium perfringens* (Shahidi-Ferguson Perfringens (SFP) Agar, Oxoid CM587) and *Staphylococcus aureus* (Baird parker Agar Base (BP), Oxoid CM275) were determined by the spread plate method. Suspect *Staphylococcus aureus* colonies were subjected to the coagulase test (staphylase test, Oxoid DR595) and API Staph test (Biomerieux 20 500) to confirm identification. *Escherichia coli* was determined by the pour plate method using Violet Red Bile Agar (VRBA, Oxoid CM107).

Results and Discussion

A summary of environmental conditions in Ngaoundere, Cameroon during these studies is presented in **table 1**. A summary of results for trial 1 is presented in **table 2**. Moisture and water activity results indicate Kilishi is sufficiently dried to minimise microbial growth. Ash levels were high when compared to fresh meat which usually contains around 3.5% mineral components when expressed on dry weight basis (fresh meat contains 1% minerals on wet weight basis). High ash levels suggest the presence of sand or dirt and also reflect the condiments in the ingredients. Protein results demonstrate the value and potential of Kilishi as a high protein food product, however the production process has been reported to lead to the loss of some soluble protein (Mbofung, 1993). The pH values for Kilishi were below the maximum accepted limit of 6.0 suggested by Pearson (1968c) for fresh meat which suggests the meat was produced from well nourished and rested stock.

Fat is extremely important in flavour development of meat. As meat ages the fat deteriorates through microbial attack and tissue enzyme activity which causes the development of free acidity and oxidation of unsaturated bonds. This results in the development of bad odours and deterioration of taste. Pearson (1968b) reported that unpleasant flavours in cooked beef were first noticeable at a level of 2-3% (as oleic acid) in extracted fat. FFA values in meat progressively inreased with storage time and Pearson (1968b) stated that for odour to be acceptable the FFA should not exceed 1.2%. FFA levels for Kilishi exceeded these limits. The presence of groundnut oil may add to the flavour of the product, but its high fat content may have had a negative effect on the quality of the product as it is suspected that poor quality oil was used in the preparation of the meat product. However, it is thought

Table 1. Environmental conditions at Ngaoundere during the months of November and December

Environmental conditions*	Day	Night
Temperature (°C)	31.0 ± 0.1	13.2 ± 0.3
Relative humidity (%)	24.8 ± 1.2	83.6 ± 1.9

*Mean ± S.E ; n= 42

Table 2. Summary of results for trial 1: Quality characteristics of traditional Kilishi 2 days post production

Variable	Mean ± S.E. **
Moisture (%)	6.92 ± 0.55
Water activity*	0.59 ± 0.02
Ash (%)	6.72 ± 0.13
Protein (%)	61.96 ±1.85
pH	5.81 ± 0.06
Fat (%)	25.39 ± 1.65
Free fatty acids (%) of extracted fat*	4.34 ± 0.43
Calcium (mg/100g)	55.69 ± 7.23
Magnesium (mg/100)*	123.98 ± 13.93
Phosphorus (mg/100g)*	392.42 ± 59.88
Calcium/Phosphorus (Ca: P) ratio	0.14 ± 0.02
Aerobic plate count (cfu/g)*	$7.4 \times 10^4 \pm 9.4 \times 10^3$
Moulds and yeast (cfu/g)*	$6.1 \times 10^2 \pm 3.0 \times 10^2$
Xerophilic moulds (cfu/g)*	$2.6 \times 10^3 \pm 7.3 \times 10^2$

For mean, n = 8
** Standard error
* = analyses on wet weight basis (WWB); all others on dry weight basis (DWB)

Table 3. Summary of results for trial 2: Quality characteristics of Kilishi during short-term storage using potassium sorbate as an anti-fungal agent

Variable		Elapsed time (weeks)			
		0	1	2	3
Moisture content (%)	(c)	9.71	9.13	8.21	7.93
	(t)	9.71	8.00	7.06	5.76
Water activity	(c)	0.80	0.81	0.78	0.70
	(t)	0.80	0.76	0.65	0.59
pH	(c)	5.55	5.62	5.88	5.90
	(t)	5.55	6.02	6.09	6.07
FFA (%) of extracted fat	(c)	2.43	3.78	4.85	5.71
	(t)	2.43	3.66	3.71	4.59
Aerobic plate count*	(c)	7.1×10^4	1.0×10^6	1.2×10^6	1.9×10^6
	(t)	7.1×10^4	6.5×10^5	8.3×10^5	1.2×10^6
Moulds and yeasts*	(c)	$< 1.0 \times 10^2$	1.8×10^2	1.1×10^2	2.7×10^2
	(t)	$< 1.0 \times 10^2$	$< 1.0 \times 10^2$	$< 1.0 \times 10^2$	$< 1.0 \times 10^2$
Xerophilic moulds*	(c)	$<1.0 \times 10^2$	1.4×10^2	1.2×10^2	2.9×10^2
	(t)	$<1.0 \times 10^2$	$< 1.0 \times 10^2$	$< 1.0 \times 10^2$	$< 1.0 \times 10^2$

Mean, n =2; (c) = Control; (t) = Treatment; * = cfu/g
All analyses on wet weight basis (WWB)

Table 4. Summary of results for trial 3: Quality characteristics of Kilishi during long-term storage using potassium sorbate as an anti-fungal agent

Variable	Control	Treatment1	Treatment 2
Aerobic plant count (cfu/g)	1.2×10^5	2.7×10^3	7.7×10^4
Moulds and Yeast (cfu/g)	1.4×10^3	$< 1.0 \times 10^2$	2.0×10^3
Xerephilic moulds (cfu/g)	4.4×10^3	$< 1.0 \times 10^2$	3.1×10^3
Clostridium prefringens (cfu/gm)	6.6×10^2	$<1.0 \times 10^2$	$<1.0 \times 10^2$
Water activity	0.56	0.39	0.43
pH	6.5	6.3	6.40
Aflatoxin B_1 (µg/kg)	113.10	1.40.65	130.05
Aflatoxin B_2 (µg/kg)	nd	nd	nd
Aflatoxin G_1 (µg/kg)	81.10	73.55	88.95
Aflatoxin G_2 (µg/kg)	nd	nd	nd
Total aflatoxin (µg/kg)	194.30	178.20	219.00

Mean, n= 2
Nd = not detected
All analyses on wet weight basis (WWB)

that unpleasant flavours and odours would be difficult for consumers to detect due to the spicy nature of Kilishi.

Calcium and magnesium results were similar to those obtained by Mbofung (1993) who demonstrated that the mineral content of Kilishi was higher than that of fresh beef (Ca 50.47 and Mg 68.12mg/100g). Phosphorus results were also higher than levels typically found in fresh beef (276.00mg/100g) (Lawrie, 1979). This increase is reflected by an increase in ash content and is mainly due to moisture loss. Mbofung (1993) found that although relative amounts of minerals were higher in Kilishi than in fresh beef, their relative solubility was lower in Kilishi. Processing of beef into Kilishi appears to lead to a decrease in the availability of its calcium and magnesium. Mbofung (1993) demonstrated the negative effect of seasoning, sun drying and roasting on the availability of calcium (approx 60% reduction) and magnesium (approx 40% reduction). The Ca:P ratio for Kilishi falls below the accepted range of 0.5-2.0 (Recommended dietary allowances, 1980). However, Kilishi is generally seen as a snack food rather than as an essential part of the diet and the impact of reduction in mineral availability will be minimal but may require further investigation.

No significant growth ($<1.0 \times 10^2$ cfu/g) of *E. coli; Staphylococcus aureus or Clostridium perfringens* was observed. Aerobic plate count results were acceptable when compared to suggested limits of 2.5×10^5 to 1.0×10^8 cfu/g (Pearson 1968a). The results for moulds, yeasts and xerophilic moulds indicate significant ($< 1.0 \times 10^2$ cfu/g) but low levels of growth. A summary of results for trial 2 is presented in table 3. The reduction in moisture content and water activity between treated and control samples of Kilishi after 3 weeks storage is thought to be due to the heat processing stage of the potassium sorbate treatment system. General reduction in moisture content and water activity (control and treatment) is not necessarily conducive to the quality or organoleptic acceptability of Kilishi. The initial moisture content and water activity was not as low as those observed in trial 1 indicating variation in production standards. The increase in pH levels for Kilishi (treatment) observed during the storage period is thought to be influenced by the potassium sorbate treatment. The reduction in the rising FFA levels for Kilishi (treatment) over the storage period indicate a higher degree of oxidative stability. Nigerian Kilishi analysed by Igene (1988) reached unacceptable levels of fat spoilage after approximately 3 weeks, according to limits (Thiobarbituric acid number (TBA) = 1.8) suggested by Pearson (1968b). A TBA number of 1.8 would correspond to a FFA value of 1.2-2.1%. Results here suggest a higher degree of fat rancidity in Cameroonian Kilishi than Nigerian Kilishi. However, the Kilishi examined by Igene (1988) was made using defatted groundnut.

No significant growth ($<1.0 \times 10^2$ cfu/g) was observed for *E. coli, Staphylococcus aureus or Clostridium perfringens* over the storage period. Aerobic plate count results fall within accepted limits (Pearson 1968a) throughout the storage period, with lower levels of growth in the treated Kilishi than in the control. There were significant levels of growth ($>1.0 \times 10^2$ cfu/g) of moulds, yeasts and xerophilic moulds in the control Kilishi and an absence of growth in the treated Kilishi. These results generally suggest that treatment of Kilishi with potassium sorbate and polythene packaging will confer a degree of product protection from mould contamination. Potassium sorbate is known to be more effective against moulds than bacteria and at pH values of 6.5 or less (Banwart, 1989) and is confirmed by these results. These properties make it suitable for use on dried meat products such as Kilishi.

A summary of results for trial 3 is presented in table 4. Aerobic plate count results for the control were similar to those observed for Kilishi in week 0 (table 2) demonstrating product stability. Aerobic plate count results were markedly lower in both treatments, especially

treatment 1. Growth levels of moulds in the control were similar to those observed in week 0 (**table 2**). However, similar growth levels were also observed in treatment 2 indicating that simple polythene packaging does not inhibit mould growth. No significant mould growth ($<1.0 \times 10^2$ cfu/g) was observed in treatment 1, suggesting that the potassium sorbate treatment system was responsible for the complete inhibition of mould growth in Kilishi. After 8 weeks storage the first significant ($>1.0 \times 10^2$ cfu/g) *Clostridium perfringens* growth was observed in the control, however no significant growth was observed in either treatment. The pH levels for the control had continued to rise from levels observed in trials 1 and 2. However, pH levels in treatments 1 and 2 were similar to those observed in trial 2 suggesting a degree of stability. Aflatoxin results were much higher than any recommended maximum levels. In the USA, the recommended maximum level of aflatoxins in foods is currently 20 μg/kg, whereas in the UK 4μg/kg is the accepted maximum level (Anon, 1993). However, much of Africa, parts of the Middle East, South America and Asia do not have legislation for aflatoxins in foods (Van Egmond, 1995). Low and stable levels of mould growth throughout the storage period indicate that Kilishi is not susceptible to excessive mould contamination. It is thought that high aflatoxin levels arose from the groundnut which accounts for about 35% of the ingredients and that any mycotoxigenic moulds which may have been present in the marinade would have been killed or inhibited by the Kilishi production process. The production process is thought to have no marked effect on existing levels of afloxins and poor quality groundnut is probably the cause of aflatoxin contamination.

Acknowledgement

The authors gratefully acknowledge the British Department for International Development for funding this work. Thanks are also due to the Institute of Agricultural Research for Development, Yaounde, Cameroon and the University of Ngaoundere, Cameroon for their invaluable cooperation and assistance.

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