Effects of Season on the Microbiological Quality of Kilishi, a Traditional Cameroonian Dried Beef Product

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Keywords: Dried meat- Micro-organism- Kilishi- Season- Quality- Cameroon

Summary

The microbiological quality of Kilishi, a traditional Cameroonian dried meat, produced in the Northern part of the country, was studied over one-year period. 79 Kilishi samples collected at various selling points were used for microbiological evaluation. The results on the microbiological analyses were then subjected to a statistical analysis using the General Linear Model (GLM) approach to assess environmental factors that affect quality. Final results indicated that the quality of Kilishi was greatly affected (P< 0.001) by the season and location of production though the total bacterial, mould and yeast counts (cfu/g) were lower than the recommended acceptability limit for the total viable bacterial counts of micro-organisms in meat at the point of consumption.

Résumé

Effets de la saison sur les qualités microbiologiques de Kilishi, une viande sèche traditionnelle du Cameroun

La qualité microbiologique de Kilishi, une viande traditionnelle sèche, produite dans le nord du Cameroun a été étudiée pour une période d'un an. 79 échantillons de Kilishi collectés à des différents points de vente ont été utilisés pour des évaluations microbiologiques. Les résultats obtenus à partir de ces évaluations microbiologiques ont été soumis à des analyses statistiques en utilisant le General Linear Model (GLM) pour déterminer les facteurs environnementaux affectant la qualité du produit. Les résultats finaux ont montré que la qualité de Kilishi a été hautement affectée (P< 0,001) par la saison et la localisation de production malgré le fait que les taux de bactériens et moisissures (cfu/g) étaient au-dessous des limites acceptables récommandées pour les taux bactériens viables de microoganismes dans la viande à la consommation.

Introduction

Due to rapid population growth within the rural and urban sectors in Cameroon, and also due changes in dietary habits of most Cameroonians, the demand for meat is on a constant increase. Meat, however, is a highly perishable food item due to abundance of a number of nutrients that favour the establishment, growth and multiplication of micro-organisms. The presence of some of these organisms in meat may render it poisonous and unfit for human consumption. As meat deteriorates very rapidly, man has over the decades developed a number of meat preservation techniques that can maintain its stability and increase its shelf-life. A major technique whose use dates back to records from the 12th century is sun drying. It was transferred to West Africa by the medieval Arabic sources (1). By this method, the moisture content of the meat is lowered to a point where the activities of food spoilage and food poisoning micro-organisms are inhibited (15). This process however, is not *per se*, lethal to many micro organisms; especially in situations where the meat is of poor quality. Today, a variety of sun-dried meat products exist. They include amongst other; the Pemmican that is prepared by the North American Indians by exposing strips of lean meat to the sun; the Charqui, which is native to South America and Biltong, found in South Africa, prepared by salting followed by air-drying (11). In Cameroon, the dried meat product Kilishi, constitutes one of daily delicacies and is found in large quantities on the local markets, streets, food stores and many households.

Kilishi is prepared mainly in the Northern sector of Cameroon, where the dry and rainy seasons fluctuate seriously. The dry season extends from October to March in the Adamawa Region and from October to May in the North and Far North Regions. The rainy season runs from April to September in the Adamawa and from June to September in the North and Far North regions. The North and Far North regions are usually characterised by very dry winds, high temperatures and low humidity. In the dry season, the air is always polluted with dust particles, carrying adhering micro-organisms which can cause important human diseases. The rainy season comes with variable violent winds, high atmospheric moisture that adversely affects health. These variable seasonal conditions, favour the

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Received on 27.05.09 and accepted for publication on 18.11.09.

spread and proliferation of micro-organisms. These, added to the questionable hygienic conditions under which the Kilishi is prepared, the questionable source of the meat, utensils, ingredients and any other materials used for its preparation, leaves a lot of concern as to its sanitary quality. It is necessary to assess the impact of season on the microbiological quality of the finished product with emphasis on the prevalence of micro-organisms such as *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Clostridium perfringens*, (*C. perfringens*) yeasts and moulds, alongside the constraints mentioned above. The aim of the study is therefore to assess sanitary quality of Kilishi as to determine whether it meets the acceptable limit (13) for the total viable bacterial (TVB) counts of micro-organisms at point of consumption.

Materials and methods

Preparation of Kilishi

The product which is not standardized is prepared by marinading (mixing ingredients of groundnut cake, salt, sugar, onion, garlic peppers etc.) very thin sheets of lean meat in slurry of ingredients, sun drying and briefly roasting over a fire by the Fulani and Hausa tribes. The pH of the production varies from 5.8; two days post production to 6.4, for long storage duration (9). The stages involved in its preparation vary among producers but involve principally the skilful cutting of quality lean meat into thin sheets of about 1- 2 mm thick. These sheets are sun-dried on raised wooden surfaces covered in rush matting followed by immersion in a slurry of groundnut sauces. It is seasoned with sugar, salt and spices for one hour and then sun-dried again for about five -12 hours and briefly roasted over a fire (7, 12).

Samples for the study

A total of 79 Kilishi samples (41 for the dry season and 38 for the rainy season) were randomly bought under natural conditions of sale, at various selling points in the three Northern Regional Headquarters; Ngaoundere in the Adamawa, Garoua in the North, and Maroua in the Far-North, twice monthly. Collected samples were wrapped in the traditional brown papers and placed in polystyrene bags and then transported in coolers to the Veterinary Research Laboratory at Wakwa-Ngaoundere, Cameroon where they were immediately placed in the deep freezer to avoid any microbial growth. The next day, all samples were removed from the freezer, placed on the laboratory bench and allowed to return to room temperature for microbial analyses to begin.

Sample preparation and analysis

About 30 g of each sample were aseptically weighed and macerated by use of a sterilised and chilled waring blender (MPR104-D15R, UK) and then subjected to a range of microbiological tests to determine its quality. The 30 g of each macerated Kilishi was mixed with 270 ml of sterile maximum recovery diluent (Oxoid, CM 733) and macerated for one minute at low speed and one minute at high speed in a stomacher (Seaward Medical, UK). Serial solutions were made up to 10^{-5} using sterile recovery diluent, universal bottles and pipettes. Selective media (Table 1) were prepared according to the manufacturer's instructions and used to culture for the presence of the micro-organisms.

Duplicate plates were prepared for each dilution and after incubation; organisms were counted using a colony counter (CNW-334 - 532G Gallen Kamp, U.K). Suspect *S. aureus* colonies were subjected to a coagulase test (Staphylase test Oxoid DR 597) and the Analytical Profile Index (API - Staph Biomérieux 20500) test to confirm identification. The results of the counts were subject to a statistical analysis (14) using a General Linear Model (GLM) and presented thus;

$$Y_{ijk} = \mu + L_i + S_j + (L*S)_{ij} + e_{ijk};$$

Where Y_{ijk} represented the counts of viable bacterial,

Table 1
Microbiological culturing and examination of organisms

Micro-organisms	Selective medium used	Method of culture*	Incubation temp. ^{0}C	Duration of incubation
Total viable bacterial counts (TVB)	PCA (Oxoid CM 463)	Spread plate	35	3-5 days
Staphylococcus aureus	BP (Oxoid CM 275)	Spread plate	37	24 hours
Clostridium perfingens	SFP (Oxoid CM 587)	Spread plate	37	24 hours
Escherichia coli	VRBA (Oxoid CM 107)	Pour plate	35	24 hours
Moulds and yeast counts	DRBC (Oxoid CM 727)	Spread plate	22-25	3-5 days
Xerophilic moulds	DG 18 (Oxoid CM 799)	Spread plate	22-25	3-6 days

Pca: Plate count agar; BP: Baird parker; SFP: Shahidi Fergason; VRBA: Violet red bile agar; DRBC: Dichloran Rose Bengal Chloramphenicol agar; DG18: dichloran glycerol 18 agar. *Method of Harrigan and McCannce 1976 (5).

Table 2
Effect of location and season on quantified microbiological contents of Kilishi with respect to Least Square Means (LSM) and Standard Error (SE) on bacteria counts

Effect	Viable bacterial counts	*S. aureus*	*C. perfingens*	*E. coli*	V Counts
μ	4.0×10^5	0.99×10^2	1.15×10^2	0.02×10^2	3.53×10^4
Location	***	***	***	ns	***
Garoua	$9.63 \times 10^5 (2.7 \times 10^4)^a$	$0.84 \times 10^2 (0.06 \times 10^2)^a$	$1.43 \times 10^2 (0.06 \times 10^2)^a$	$0.02 \times 10^2 (0.005 \times 10^2)^a$	$2.72 \times 10^4 (1.50 \times 10^3)^a$
Maroua	$1.22 \times 10^5 (2.9 \times 10^4)^b$	$0.83 \times 10^2 (0.06 \times 10^2)^a$	$0.76 \times 10^2 (0.06 \times 10^2)^b$	$0.02 \times 10^2 (0.01 \times 10^2)^a$	$3.07 \times 10^4 (1.58 \times 10^3)^a$
Ngaoundere	$1.93 \times 10^5 (2.54 \times 10^4)^b$	$1.28 \times 10^2 (0.05 \times 10^2)^b$	$1.26 \times 10^2 (0.06 \times 10^2)^a$	$0.02 \times 10^2 (0.01 \times 10^2)^a$	$4.83 \times 10^4 (1.39 \times 10^3)^b$
Season	***	***	***	***	***
Dry	$3.91 \times 10^4 (2.18 \times 10^4)^a$	$0.54 \times 10^2 (0.05 \times 10^2)^a$	$0.63 \times 10^2 (0.05 \times 10^2)^a$	$0.00 \times 10^2 (0.01 \times 10^2)^a$	$9.79 \times 10^3 (1.19 \times 10^3)^a$
Rainy	$8.13 \times 10^5 (2.28 \times 10^4)^b$	$1.43 \times 10^2 (0.05 \times 10^2)^b$	$1.68 \times 10^2 (0.05 \times 10^2)^b$	$0.04 \times 10^2 (0.01 \times 10^2)^b$	$6.09 \times 10^4 (1.20 \times 10^3)^b$
Location* season	***	***	***	***	***
Garoua *dry	$2.69 \times 10^4 (3.7 \times 10^4)$	$0.48 \times 10^2 (0.08 \times 10^2)$	$0.66 \times 10^2 (0.08 \times 10^2)$	$0.00 \times 10^2 (0.01 \times 10^2)$	$6.64 \times 10^3 (2.03 \times 10^3)$
Garoua *rainy	$1.90 \times 10^5 (4.02 \times 10^4)$	$0.12 \times 10^3 (0.08 \times 10^2)$	$0.22 \times 10^3 (0.09 \times 10^2)$	$0.03 \times 10^2 (0.01 \times 10^2)$	$4.77 \times 10^4 (2.20 \times 10^3)$
Maroua *dry	$1.58 \times 10^4 (4.02 \times 10^3)$	$0.56 \times 10^2 (0.08 \times 10^2)$	$0.52 \times 10^2 (0.09 \times 10^2)$	$0.00 \times 10^2 (0.01 \times 10^2)$	$4.08 \times 10^3 (2.20 \times 10^3)$
Maroua *rainy	$2.30 \times 10^5 (4.19 \times 10^4)$	$0.11 \times 10^3 (0.09 \times 10^2)$	$1.00 \times 10^2 (0.10 \times 10^2)$	$0.01 \times 10^2 (0.01 \times 10^2)$	$5.74 \times 10^4 (2.30 \times 10^3)$
N'dere *dry	$7.46 \times 10^4 (3.59 \times 10^4)$	$0.56 \times 10^3 (0.07 \times 10^2)$	$0.71 \times 10^2 (0.08 \times 10^2)$	$0.00 \times 10^2 (0.01 \times 10^2)$	$1.87 \times 10^4 (1.96 \times 10^3)$
N'dere *rainy	$3.11 \times 10^5 (3.59 \times 10^4)$	$2.00 \times 10^3 (0.07 \times 10^2)$	$1.81 \times 10^2 (0.08 \times 10^2)$	$0.05 \times 10^2 (0.01 \times 10^2)$	$7.19 \times 10^4 (1.97 \times 10^3)$

Mean values within the same column for the same effect with different superscripts differ significantly at P< 0.001 differently, NS= 0.05, N'dere= Ngaoundere; V counts= mean of viable bacteria, *S. aureus, C. perfingens,* and *E. coli*.

Table 3
Location and seasonal impact on the microbiological quality of Kilishi with respect to mean counts x 10^2 and standard errors x 10^2 on fungi

Effect	Moulds and yeast	Xerophilic moulds	V Counts
μ	1.00	0.75	1.15
Location	***	***	***
Garoua	0.72 (0.05)^a	$0.86 \times 10^2 (0.05)^a$	0.76(0.05)^a
Maroua	1.49 (0.06)^b	$0.68 \times 10^2 (0.06)^b$	1.08(0.06)^b
Ngaoundere	0.96 (0.05)^c	$0.78 \times 10^2 (0.05)^b$	0.87(0.05)^c
Season	***	***	***
Dry	0.18 (0.04)^a	$0.15 \times 10^2 (0.04)^a$	0.17(0.04)^a
Rainy	1.93 (0.05)^b	$1.40 \times 10^2 (0.04)^b$	1.65(0.04)^b
Location* season	***	***	***
Garoua *dry	0.15 (0.07)	$0.13 \times 10^2 (0.04)$	0.14(0.07)
Garoua *rainy	1.21 (0.09)	$1.60 \times 10^2 (0.08)$	1.38 (0.09)
Maroua *dry	0.18 (0.08)	$0.16 \times 10^2 (0.08)$	0.17(0.07)
Maroua *rainy	2.80 (0.08)	$1.20 \times 10^2 (0.08)$	2.00 (0.08)
Ngaoundere *dry	0.22 (0.07)	$0.17 \times 10^2 (0.07)$	0.20 (0.07)
Ngaoundere *rainy	1.70 (0.07)	$1.40 \times 10^2 (0.07)$	1.55 (0.07)

V counts= mean of moulds and yeast and xerophilic moulds; mean values within the same column for the same effect with different superscripts differ significantly at P< 0.001.

S. aureus, *C. perfingenes*, and *E. coli*, moulds and yeast, *xerophilic* moulds of the k^{th} sample;
μ represented the overall mean;
L_i representing the effect of the i^{th} location (I= Ngaoundere, Garoua, Maroua);
S_j, the effect of the j^{th} season (j= dry and rainy season);
$(L^*S)_{ij}$, the effect of the first order interaction of i^{th} location by j^{th} season and
e_{ijk} random error associated with counts of viable bacterial, *S. aureus*, *C. perfingens*, moulds and yeast and *xerophilic* moulds for the k^{th} sample collected from the l^{th} location during the j^{th} season.

Results

The major micro-organisms found in Kilishi comprised of viable bacteria, *S. aureus*, *C. perfingens*, *E. coli*, moulds and yeast, x*erophilic* moulds (Table 1).
The effects of location and first order interaction between location and season on quantified microbiological contents of Kilishi are shown in table 2.

The effect of locality on counts of the various organisms (on the culture media) was highly significant (P< 0.001), except for E. *coli*. Total viable bacteria counts on Kilishi obtained on samples from Garoua was significantly (P< 0.001) higher than those obtained on Kilishi from Ngaoundere and Maroua. *S. aureus* counts on samples from Ngaoundere were significantly higher (P< 0.001) than those from Garoua and Maroua which registered no significant difference (P> 0.05). Counts for *C. perfringens* were significantly higher (P< 0.001) on samples obtained from Garoua and Ngaoundere as compared to those from Maroua. Levels of *E. coli* on the samples were inconsequential (P> 0.05) for the three regions. The mean (V) counts of viable bacteria *S. aureus*, *C. perfingens* and *E. coli* for samples obtained from Ngaoundere were significantly higher (P< 0.001) than those obtained from Maroua and Garoua. Though the level for samples obtained in Maroua was higher than that obtained in Garoua, there was no significant difference (P> 0.05). Counts of all micro-organisms obtained rainy season samples were significantly higher (P< 0.001) than those obtained in the dry season in the three regions. This is confirmed with the levels of significance (P< 0.001) obtained in the first order interaction between location and season.

The effect of location, season and interaction between season and location on the microbiological quality of Kilishi with respect to counts of fungi, are presented in table 3.
The presence of moulds and yeasts was significantly (P< 0.001) affected by the location and season. Counts of moulds and yeasts on samples from Maroua were significantly (P< 0.001) higher compared to those obtained on samples from Garoua and Ngaoundere. Xerophilic moulds were significantly (P< 0.001) higher for Garoua samples compared to Maroua and Ngaoundere whose values were not significantly different (P> 0.05). Average (V) counts were also affected by location. Mean registered for Maroua was higher and significantly (P< 0.001) different from those registered for Ngaoundere and Garoua. However, mean value obtained for Ngaoundere was significantly higher than that obtained from Garoua.

Counts of fungi obtained on rainy season samples were significantly (P< 0.001) higher than those obtained on samples collected during the dry season. This observation is confirmed by mean counts obtained for the interaction between location and season.

Discussion

The extent to which a product is contaminated by micro organisms depends on the level of hygiene and sanitation of persons involved and material used in the production chain. The degree of humidity of a food material is responsible for the initiation or inhibition of the growth of micro-organisms. Traditionally dried or low moisture foods are those which contain not more than 25% moisture and water activity (a_w) of 0.0 to 0.60 (8). Bacteria require relatively high levels of moisture for growth. Yeasts would require less and moulds, still less quantity. The moisture content and water activity attributes of Cameroonian Kilishi have been studied (9). They put the levels at 6.92% and 0.59 respectively. These levels indicate Kilishi to be a very dry and stable quality product. Also, treating Kilishi with 10% (w/v) potassium sorbate and, proper polythene packaging will confer some degree of protection from contamination by moulds (3).

In this report, the minimum detection limit of growth of micro-organisms is considered to be 1.0×10^2 cfu/g. Samples having growth levels below this limit are regarded as having “no significant growth”. There has been a lot of debate concerning the acceptability limit for the total viable bacteria (TVB) counts of micro-organisms in meat at the point of consumption. Pearson (13) puts the limit between 2.5×10^5 to 1.0×10^8 cfu/g of consumable meat. Tables 2 and 3 show the aerobic plate count on PCA for Kilishi consumption for the two seasons and the results indicate significant levels of growth ($>1.0 \times 10^2$ cfu/g). These levels are acceptable when compared to the limit indicated above. The level of growth was higher in the rainy season than in the dry season for the three regions. This may be due to an increase in the relative humidity in the atmosphere which could have caused the dry product to pick some moisture to cause the growth of some micro-organisms, especially if the product was not well preserved. The dry season values for Ngaoundere samples, which is located on the Adamawa plateau, were generally

higher than those for Garoua and Maroua samples. This may be explained by the fact that the dry season in the Adamawa is shorter and characterised by a cooler weather than that in the North and Far North regions that are situated on the low-lying altitude. The dry seasons are of longer duration, characterized by dry and hot weather conditions with occasional dusty winds. The rainy seasons are of short duration.

The degree of meat spoilage is usually influenced in part, by the microbial load at the beginning of production, packaging and handling of the finished products. Aerobic plate counts (Tables 2 and 3) indicate that the level of microbial growth for the finish product during the dry rainy season is acceptable following the criteria set by Pearson (13). With the exception of *E. coli*, (< 1.0 x 10^2 cfu/g), there were higher levels of bacteria growth in the rainy season. In the dry season, there was complete absence of *E. coli* and there was neither significant (P> 0.05, <1.0 x 10^2 cfu/g) difference of the growth of *S. aureus* and *C. prefringens* for all the three regions. This indicates that, for significant level of bacteria to establish and grow on Kilishi, it will need some levels of moistures. This can only be achieved if the product was not properly packed and exposed to or stored under conditions of relatively high humidity. For S*. aureus* to grow it would need a_w of 0.86 and *C. perfringens* and *E. coli* would not grow below a_w of 0.95 and 0.96, respectively (4). These levels are greater than that of Kilishi (a_w 59) (9). In the rainy season, there was significant (>1.0 x 10^2 cfu/g) but low levels of growth of these organisms. This may be due to increase of moisture levels, poor weather and poor conditions of handling the product that are usually abound during this time of the year.

The result of moulds, yeasts, and xerophilic moulds (Table 3) indicate that the levels of growth were significantly higher than, 1.0 x 10^2 cfu/g for both seasons with a slight growth increase in the rainy season. The levels were lower than those of the bacteria and were acceptable when compared to the limits of 2.5 x $l0^5$ to 1.0 x 10^8 cfu/g suggested by Pearson (13). This is an indication that, Kilishi is not pruned to excessive contamination by these organisms. Important fungi isolated in low numbers included *Candida* and *Aspergillus spp*. Most, if not all of these might have arisen from air borne dust circulating in the atmosphere among other sources. In general, the levels of growth of micro-organisms in both seasons were low and though those of the rainy season were higher, they felt within the level of acceptability set by Pearson (13).

Some spices used in Kilishi production play a key role in inhibiting the growth and proliferation of some micro-organisms. It was reported that extracts of garlic, for example have been shown to inhibit many species of fungi (2), onions and ginger (6) have been shown to prevent or greatly reduce the development of rancidity and oxidative flavour deterioration (10). Their incorporation in the Cameroonian Kilishi could be accountable for its good quality, flavour and palatability.

Conclusion

Kilishi may therefore be a stable dried meat product capable of imposing a barrier to the establishment and multiplication of significant levels of micro-organism. Due to its high microbiological quality, it is safe to consume the product throughout the year regardless of the season and place of production. However, good precautionary measures must be taken to prevent or greatly reduce its contamination by micro-organisms, especially during the rainy season. To achieve this, technical controls in many respects are necessary. Only meat of good microbiological quality should be used for processing. During transportation of carcass and preparation of the product, measures should be taken to avoid contamination. Pieces of meat to be used must be thin enough to ease fast drying in order to minimize the growth of external mesophilic microbial contaminants. The final product must be dried to a sufficiently low water content relative to lean content and protected by employing suitable packaging techniques to avoid re-absorption of moisture.

Acknowledgement

We are grateful to the British Department for International Development for supplying some of the materials used for this work.

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AVIS

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Nous pensons ainsi, grâce à votre aide, pouvoir rendre un grand service à la communauté pour laquelle vous travaillez.

Merci.

BERICHT

Wij herrineren al onze lezers eraan, vooral diegenen in de ontwikkelingslanden, dat TROPICULTURA bestemd is voor ieder die werk verricht op het gebied van het platteland en dit in de meest ruime zin van het woord.

Daarom zou het nuttig zijn dat u ons de adressen zou geven van de Instellingen, Scholen, Faculteiten, Centra of Stations voor landbouwonderzoekvan het land of de streek waar U zich bevindt. Wij zouden ze kunnen abonneren, zo dit niet reeds gebeurd is.

Met uw hulp denken we dus een grote dienst te kunnen bewijzen aan de gemeenschap waarvoor u werkt.

Dank U.