Effect of Temperature, Storage Containers and Improved Hygiene on Microbial Safety and Chemical Quality of Traditional Meat Products

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ABSTRACT

Preservation of meat and meat products is important due to its short shelf life and perishability. The pastoralists of Northern Kenya processed and preserved traditional meat products for consumption. The traditional meat products are relished food both for nutritional and cultural heritage among the Borana pastoralists. The study was carried out to assess the microbial safety and chemical quality of traditional meat products. The samples were collected immediately after processing and transported in a cool box to the food laboratory for microbial analysis and determination of changes related to lipid oxidation during storage for seven weeks at ambient temperature and at refrigerated temperature (5 °C). Microbiological quality of the samples was assessed by Total Viable Count (TVC), *Escherichia coli* count, *Staphylococcus aureus* count and yeast and mold count. Result showed that *Staphylococcus aureus*, yeasts and molds were detected in the products and there was increase of *Staphylococcus aureus* count from (1.44 log₁₀ CFU/g) to (2.28 log₁₀ CFU/g) during storage at ambient temperature for seven weeks. Samples stored at refrigerated 5°C showed less counts of microbial load. The peroxide value, acid value and thiobarbituric acid levels were below the value associated with meat spoilage during the expected shelf life. Reduction of moisture during drying of traditional meat products and cooking of meat at high temperature contributed towards reduction of microbial load. However, poor handling and post contamination may lead to poor microbial quality of traditional meat products.

Keywords: meat products, microbial, quality, safety, traditional.

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I. INTRODUCTION

The Borana women prepared traditional meat products from beef or goat meat, which was striped, dried, and deep fried. It has a high cultural and economic importance to Borana people which explains the efforts to ensure quality and microbial safety. The processing and preparation of these traditional meat products was an elaborate process where the meat parts used were selected carefully by removing fatty tissues, ligaments and only lean meats parts were used. The meat was then striped and suspended to dry for one to three days to lower the moisture content. According to [1] meat products, when not consumed immediately, are often processed using a range of traditional techniques involving salting, drying, cooking, smoking, and marinating, or a combination of these operations to lengthen their shelf life.

Due to handling and preparation environment of traditional meat products, microbial contamination can arise from post handling even after high temperature has been used to kill the microorganisms during processing. Food handlers can act as vectors for food contamination leading to the transmission of enteric and respiratory pathogens to food, the

composition of the residual predominant microbiota in food products is also known to be determined by the handling, processing, and storage conditions. Interaction of all these predisposing factors could explain the principal bacterial populations associated with biltong [2].

Raw meat is a perishable product which is prone to spoilage and deterioration. It is important to observe hygiene from the point of slaughter through all stages of processing to storage to maintain high quality and safety. There are three main mechanisms for meat and meat products spoilage these are microbial spoilage, lipid oxidation and autolytic enzymatic spoilage [3]. Meat used for drying amongst pastoralists is usually derived from fresh carcasses Traditional dried meat are usually cut into slice and sun dried Reducing the moisture content of the meat is achieved by evaporation of water from the peripheral zone of the meat to the surrounding air and the continuous migration of water from the deeper meat layers to the peripheral zone [4]. Similar traditional meat product such as pemmican was dried in the sun and pounded or shredded prior to being mixed with the melted fat. This preserving method is based on the air exclusion provided by the fat, which reduces oxidative changes and diminishes microbial growth [5] Because

microbial quality of meat products is of public health concern all over the world it is important to determine the microbial safety of these products. Processed meat foods are more prone to contamination with pathogenic microorganisms during the various stages of processing [6] Moreover, since these products are preserved in oil, the possibility that lipid oxidation and the associated rancidity and production of harmful lipid oxidation products should be considered. Microbiological assessment on safety, storage stability, and sanitary quality of traditional meat products were carried out to analyses for the presence of some pathogens like *Staphylococcus aureus*, different microbial groups (e.g., Total Viable Counts and yeast and molds), and indicator bacteria (e.g., *E. coli* as an indicator of sanitation). The variation in bacterial count between different types of meat products could be attributed to difference of ingredients and steps involved in their formulation and preparation [7].

II. MATERIALS AND METHODS

A. Preparation of Traditional Meat Products and Collection of Samples

Meat products were striped and dried for one to three days at prevailing temperature conditions and later deep fried as shown in (Fig. 1) below. The women also prepared products under improved condition where basic hygiene of washing hands at critical stages were observed. The basic hygiene practices applied to improved products included observation of personal hygiene, washing of hands, cleaning and sanitizing of knives, pots and other utensils used.

The samples were then transported in a cool box immediately after collection to the Department of Food Science and Technology at Jomo Kenyatta University of Agriculture and Technology (JKUAT). At the laboratory samples were stored at different treatment condition, ambient temperature, and refrigerated temperature at 5°C and analysed for microbial load and rancidity indices using method [8]

Microbiological assessment was done to establish the microbial safety and chemical quality of traditional meat products as microorganisms are the main cause of spoilage in meat. Aerobic colony count was done as count of total viable bacteria employed to indicate the sanitary quality of food. Indicator organism, *Escherichia coli* was tested for any direct or indirect faecal contamination. Specific pathogen counts of *Staphylococcus aureus* was done to check presence of microorganisms that may cause food borne illness while yeast and molds counts was done as an indicator of food spoilage. Quality indices such as peroxide value, acid value, free fatty acid, Thiobarbituric acid (TBA) and pH were also assessed to evaluate development of rancidity in traditional meat products.

The conditions which were tested were time (seven weeks) bi-weekly, temperature (ambient and refrigerated 5°C), different storage containers (universal glass bottles, traditional containers, and stainless steel containers) and products prepared under improved conditions

Fig. 1. Process flow for traditional meat products.

B. Microbiological Analysis

Ten grams of each sample and 90 ml of normal sterile saline water was homogenized in a stomacher bag blender for 1-3 minutes to concentrate the suspended micro flora in them to form a food homogenate. Thereafter, different microbial flora from homogenate was isolated using several specialized isolation culture media. 0.1 ml samples of appropriate dilutions were spread on the following media for microbial count. Nutrient agar (Oxoid), MacConkey agar (Oxoid), mannitol salt agar (oxoid) and potato dextrose agar (oxoid) for the enumeration of total viable count, *E-coli*, *Staphylococcus aureus* and yeast and mold, respectively.

1) Total viable count (TVC).

For total bacterial counts, 0.1 ml of relevant dilutions was inoculated onto sterile nutrient agar plates and spread on the surface using sterile bent glass rod. Inoculated plates were incubated at 37 °C for 48 h before colonies were counted and reported as colony forming units/g (CFU/g).

2) *Escherichia coli* count

0.1 ml of relevant dilution was spread on surface of McConkey agar plates. The plates were then incubated for

24 h at 37 °C. Colonies were counted and reported as colony forming units/g (CFU/g).

3) *Staphylococcus aureus* count

0.1 ml of relevant dilutions was spread on mannitol salt agar plates using sterile bent glass rod on. The inoculated plates were incubated at 37 °C for 48 h. Colonies were counted and reported as colony forming units/g (CFU/g).

4) *Mould and yeast count*

Yeasts and mold were enumerated by the surface plate method using potato dextrose agar (PDA). 0.1ml of appropriate sample dilutions were spread onto PDA agar and incubated at 25 °C for 3-5 days. Colonies were counted and reported as colony forming units/g (CFU/g)

C. *Quality Indices*

The peroxide value, free fatty acid, pH thiobarbituric acid (TBA) and the acid value were analysed to determine the chemical quality of traditional meat products.

1) *Determination of Peroxide Value (PV)*

Two grams of sample were weighed into a glass stoppered flask. Twenty five milliliters of acetic acid- chloroform mixture (in ratio 3:2) was added and the sample dissolved. One milliliter of saturated potassium iodide (KI) solution (4 parts KI in 3 parts distilled water) was added, mixed, and placed in the dark for 10 min. Thirty milliliters of distilled water was added, mixed and followed by addition of 1ml of 1% starch indicator. The mixture was titrated with 0.01N sodium thiosulphate until the blue colour disappeared. A blank test containing all the reactants other than sample was carried out at the same time. The peroxide value of each sample was calculated as follows:

$$PV \% \left(\frac{\text{meq}}{100\text{g}} \right) = (B - A) \times 1000 \times N / W$$

where

B – 1st titre;

A – blank;

N – Normality of Na₂S₂O₄;

W – Weight of samples (g).

2) *Determination of pH*

The pH of meat was measured by mixing 5 g of sample with 20 ml of deionized water and stirred for 5 minutes and measured using pH meter (Hanna pH meter 213).

3) *Determination of free fatty acid*

Free fatty acid (% of oleic acid) were analyzed by using standard method: 2 g of sample was weighed into a 250 ml conical flask and 10ml of ethanol (95%) was added, the resulting mixture was titrated with sodium hydroxide (0.1 M) using phenolphthalein as indicator. The titration was done with constant shaking until a pink colour persisted for 30 seconds. The %FFA was then calculated from the following equation:

$$\% \text{ FFA} = (1\text{st titre} \times 282 \times N) / (F \times W)$$

where

W – Weight of sample (g);

N – Normality NaoH;

F – Factor, (10);

282 – Conversion factor for m/w (oleic acid).

4) *Determination of Acid value*

About 2.5 g of meat sample slurry was weighed out into a 100 ml beaker and 50 ml of distilled water added. The sample was then titrated with 0.1 N NaOH in the presence of phenolphthalein indicator until the end-point was reached, signified by a change of indicator colour to pink. The volume (ml) of NaOH used was recorded and used in determining % acid.

$$\text{Acid value}\% = \frac{\text{ml of NaOH} \times 0.1\text{N NaOH}}{\text{milliequivalent factor} \times 100 / (W)}$$

where

W – Weight of sample (g);

N – Normality NaoH;

M/w (oleic acid).

5) *Determination of Thiobarbituric Acid Reactive Substances (TBARS)*

Analytical Procedure: The standard malondialdehyde (MDA) solution (1 ml) was taken in a 10 mL test tube and mixed with Thiobarbituric Acid ((TBA) (1 ml). The mixture was heated in a boiling water bath at 95 °C for 60 minutes. The test tubes were cooled at room temperature and absorbance was measured at 532 nm using UV-visible spectrophotometer model PharmaSpec 1800 (Shimadzu, Japan). Each standard for the calibration was repeated according to the above procedure. A blank sample was repeated

Preparation of malondialdehyde (MDA) and Calibration Standards: Standard stock solution of MDA (1 mM) was prepared in glacial acetic acid. MDA (31.35 mg) was accurately weighed and dissolved in 100 mL solvent. From the stock solution, different concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mM were prepared. The calibration curve was constructed in the concentration range of 0.1 to 1.0 mM.

Extraction of ThioBarbituric Acid Reactive Substances (TBARS) in Samples: One gram of ground meat sample was taken in 25 mL test tube and 5 mL of the solvent. The solvent was either 100% glacial acetic acid (AA) or 50% glacial acetic acid in water (AW). The extract of each sample (1 ml) was mixed with 1 ml TBA reagent, and the above procedure was repeated five times. The TBARS was calculated using the formula as M/g of the sample: where the amount is determined from the calibration curve and the weight of the sample taken.

The TBARS was calculated using the formula in µM/g of the sample as:

$$\text{TBARS in } (\mu\text{M/g}) = \frac{Ac \times V}{W}$$

where

Ac is the amount determined from the calibration curve;

W is the weight of the sample taken;

V is volume in mL or dilution factor of the total extract prepared.

D. Data Analysis

The data obtained were subjected to analysis of variance (ANOVA) to evaluate microbial and chemical quality characteristic of traditional meat products. When significant difference was observed between the samples for a parameter, LSD (Least Significant Difference) test to identify the means that are different. Statistical significance was measured at ($P < 0.05$) using SPSS Version 19.0 software and Microsoft office excel was also used to generate tables and bar graphs. The data were expressed as mean count $\pm$ standard error of mean.

III. RESULTS AND DISCUSSIONS

A. Changes in Microbial counts during Storage at Different Temperature Conditions

Result presented in (Fig. 2) showed that there were viable microorganisms as indicated by the total viable count, *Staphylococcus aureus* and yeast and mold for the period of seven weeks observed at ambient temperature.

Fig. 2.: Changes in counts (log₁₀ CFU/g) at ambient temperature conditions observed biweekly for seven weeks.

The result further indicates that, there was significant ($p < 0.05$) increase of *Staphylococcus aureus* from (1.28 log₁₀ CFU/g) to (2.28 log₁₀ CFU/g) over the observed period at ambient temperature. However, for all the microorganisms analysed the counts were within acceptable limit as stated by [9] that require any cooked food to contain no more than 10⁶ viable counts per gram upon analysis. From all the samples tested *Escherichia coli* was not detected (ND). The presence and increase of microbial count during the time observed could be attributed to cross and post handling contamination [10]. Observed that the application of high temperature during roasting and the process of drying of the meat were expected to have reduced the bacterial load of the meat product. However, the presence of bacterial counts despite these processes suggests possible post-production contamination. Yeast and molds can cause spoilage of jerky a dried meat products during storage of the product acquires moisture from the environment [11].

Comparing the samples stored at ambient temperature and refrigerated temperature (5 °C) (Fig. 2 and 3), showed that there was decline in counts observed for total viable count, *Staphylococcus aureus* and yeast and molds in refrigerated

temperature (2.51 to 1.91 log₁₀ CFU/g); 2.28 to 0.63 log₁₀ CFU/g) and 1.74 to 1.21 log₁₀ CFU/g), respectively. This shows that temperature condition during storage do have an effect on microbial growth and keeping quality of this traditional meat products. Cool (or chill) storage refers to storage at temperatures from about 16 to -2 °C. Chemical reaction rates decrease as temperatures decrease [12].

Escherichia coli was the best faecal indicator organism to assess sanitation conditions during processing because of their high prevalence in the faeces of healthy animals [13]. The presence of *Staphylococcus aureus* and yeast and molds of counts in traditional meat products indicate that there was post cooking contamination at ambient temperature. *Staphylococcus aureus* may be present in small numbers in dried meat and is frequently introduced into foods by way of abscesses or the nasal cavity of food handler's [14]. Smoke constituents play an important role in preserving the product against microbial spoilage. [15]

Fig. 3. Changes in counts (log₁₀ CFU/g) at refrigerated temperature (5 °C) conditions observed biweekly for seven weeks.

B. Different Storage Containers and Temperature

For the storage containers, (Fig. 4) Results indicate that there were microbial counts observed in all the three-container type at ambient temperature. However, stainless-steel containers had higher counts for total viable count, *Staphylococcus aureus* and yeast and molds more than the universal glass bottle and traditional containers. This could have attributed to pre-drying of meat products and that the traditional container was fumigated and smoked by special kind of stick. It was reported pastoralist normally fumigate their food containers for preservation of meat and milk.

Result in (Fig. 5) for storage containers stored at refrigerated temperature (5°C), revealed that there was significant ($p < 0.05$) decline in growth of *Staphylococcus aureus* count for all the three containers at refrigerated temperature (5 °C).

However, there was presence of viable microorganisms and yeast, and molds counts. Although cooking destroys *Staphylococcus aureus*, the toxin it produces is heat stable. Refrigeration of raw and cooked foods will inhibit the growth of *Staphylococcus aureus* out of foods [16].

A significant difference in microbial load reduction was observed with different storage temperatures conditions and in products stored in universal glass bottles and traditional

containers. Proper handling, pre-treatment and preservation techniques can improve the quality of meat and meat products and increase their shelf life [17].

Fig. 4. Microbial counts (log₁₀ CFU/g) observed in different storage containers at ambient temperatures at week one.

A study by [18] found that aerobic plate count for jerky which is sold as heat treated ready to eat meat products was detected at levels considered safe for human consumption at 3.21 to 3.45 log₁₀ CFU/g over ambient storage of 28 days. Similarly [19] reported that biltong, a dried salted beef product in South Africa, had aerobic bacteria (6.4 to 7.0 log₁₀ CFU/g), *staphylococcus aureus* (3 log₁₀ CFU/g and *E. coli* (1 log₁₀ CFU/g counts, yeast, and mold 6 log₁₀ CFU/g.

Fig. 5. Microbial counts (log₁₀ CFU/g) observed at refrigerated temperature (5°C) at week one.

This indicates that total bacterial count, *staphylococcus aureus*, yeast and mold in traditional meat is lower at 2.51 log₁₀ CFU/g 2.28 log₁₀ CFU/g and 1.74 log₁₀ CFU/g respectively than has been reported for biltong and jerky.

C. Different Storage Containers and Improved Processing Conditions

Fig. 6 shows result of storage containers with samples prepared under traditional conditions and improved conditions where basic hygiene of washing hands at critical stages were observed. There was significant ($p < 0.05$) decline

in growth of *Staphylococcus aureus* counts for all the three storage containers in improved samples.

Fig. 6. Changes in mean counts (log₁₀ CFU/g) observed for seven weeks for product processed under improved condition.

Total viable count and yeast and molds counts were also lower in improved products [20] Described that the art of preserving meat by a combination of salting, spicing and exposure to the sun for drying has been practiced for centuries in nearly every country, especially in developing countries where refrigeration is limited or unavailable. Moisture and temperature developments during drying are indicators of the quality and safety of foods [21].

D. Effect of Different Processing Conditions on Microbial Populations

Comparing the traditional meat prepared under traditional condition by women and improved condition where basic hygiene was observed, the result in (Fig. 7) shows lower counts in all the three, total viable count at (1.23 log₁₀ CFU/g) and no counts for *Staphylococcus aureus* and yeast and molds at seven weeks. This was attributed to the basic hygiene and monitoring critical meat preparations stages (before and during) processing of traditional meat products.

Fig. 7. Changes in mean counts (log₁₀ CFU/g) in storage containers for improved products.

E. Quality Indices

Table I contains the quality indices of processed traditional meat products. There was no significant difference observed

in pH values (5.9-6.0). [23] Reported that pH of beef *kitoza* ranged from 5.26 to 6.22) similar to the pH of salted/dried beef products such as *pastirma* (5.7–6.1.) The acid value is the number of milligrams of sodium (or potassium)

hydroxide necessary to neutralize the free acids in 1 gram of sample. The acid value is a measure of the amount of free acids present in a given amount of sample.

TABLE I: pH AND QUALITY INDICES FOR TRADITIONAL MEAT PRODUCTS

Quality Indices	Beef (<i>Koche</i>)	Goat (<i>koche</i>)	Beef pounded	Mix Beef and barley
pH	5.93 ^a ±0.18	6.18 ^a ±0.08	5.71 ^a ±0.05	5.85 ^a ±0.03
Peroxide value mEq/kg	2.41 ^a ±0.81	2.56 ^b ±0.80	2.68 ^b ±0.82	1.81 ^c ±0.81
TBA mg MDA/kg	0.53 ^a ±0.01	0.42 ^b ±0.02	0.54 ^a ±0.02	0.33 ^c ±0.02
Acid value %	0.01 ^a ±0.00	0.01 ^a ±0.00	0.0 ^{0a} ±0.00	0.00 ^a ±0.00
Free fatty acid %	2.62 ^a ±0.01	1.41 ^b ±0.02	0.84 ^c ±0.02	0.14 ^d ±0.00

Means of samples analysed in triplicate ±standard error of mean. Value with different superscripts within a row were significantly different at (P<0.05).

Preventive measures can be applied at the most critical stages of preparation, storage, or display and wherever control is feasible. Home preservers should always wash their hands and sanitize utensils and work surfaces before and after processing [22] *Escherichia coli* was not detected in any samples of traditional meat products. Dried meat products are shelf stable at ambient temperature. As a loose reference to determine approximately where spoilage will occur, preserved meat should have aerobic colony count less than 6 log₁₀ CFU/g, *Escherichia coli* none detected, yeast and mould less than 3 log₁₀ CFU/g) and *Staphylococcus aureus*< 1.3 log₁₀ CFU/g) [14]. The Acid value percent was constant and there was no variation noted.

In this study, the Peroxide Value (PV) of the processed samples was 1.8–2.7 mEq/kg. These values are within the value for prevention and control of rancidity development in meat. The peroxide value is a useful method to determine the early stages of fat oxidation and the product is considered rancid when PV of 20-40 meq kg⁻¹ is reached [22].

Free fatty acids values of processed meat products were 0.14-2.62%. These values were below the threshold for rancidity detection in dried cooked meat. Thiobarbituric acid (TBA) values expressed as mg of malondialdehyde (MDA)/kg of processed products were 0.33-0.54 (MDA)/kg hence, it was within the acceptable range. TBA value is routinely used as an index of assessing lipid oxidation in meat products during storage [24] reported that the rancid flavour is initially detected in meat products at TBA values of 2.0 a threshold value of 2 mg/kg.

Peroxide value (PV) was used as indices to assess the level of lipid oxidation and it is widely used for the estimation of oxidative rancidity in fats and oils [25] There was significant increase (p<0.05) for peroxide value 2.26 to 4.45 mEq/kg at week one and week seven [26] reported significant changes in peroxide value (p<0.01) due to storage period in meat samples.

The result of quality indices, the pH, peroxide value (PV), Acid value, FFA (free fatty acid) and TBA (Thiobarbituric acid) are presented in (Fig. 8) was monitored at week one as basis to compare at week seven during storage period to observe changes in the keeping quality which shows no significant variation (p>0.05) for pH, acid value and TBA.

Free fatty acids are the products of enzymatic or microbial degradation of lipids. The mean values of the free fatty acids (% oleic acid) of samples increased significantly (p<0.05) from 0.97% to 2.05% at week 1 and week 7 respectively [27] reported that FFA level of *kilishi* during 14 days storage was about 1.8%, which implied that *kilishi* maintain a good quality at a considerably long period of storage.

Fig. 8. Changes in quality indices at week 1 and week 7.

IV. CONCLUSION

Results obtained from microbiological and rancidity indices were within acceptable range throughout the storage period. Drying and cooking of meat contributed towards reduction of microbial load. It was noted that refrigerated temperature, good hygiene practices, glass containers and traditional storage containers were practices observed to have good keeping qualities and enhanced safety for traditional meat products. However, poor handling and post contamination led to proliferation of microbes. Low moisture, low temperature, and observation of good hygiene practices prior and during processing of traditional meat products were practices observed to enhance the shelf life and quality of traditional meat products.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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