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Microbiological Status of Ready to Eat (RTE) Bovine Tripe Rolls Under Different Storage Conditions

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Abstract
Bovine tripe is a meat by-product known to favour microbial growth and can be incorporated in foodstuffs or used as a stand–alone food component. However, its utilization among sections of communities in Kenya has been limited due to inherent toughness and short-shelf-life which hinders its commercial applications. While, tripe can be the major source of microbial contamination, personnel hygiene and handling of equipment during production can be another potential source of contamination. This study was hence designed to find out the suitability and fitness for consumption of bovine tripe rolls produced from bovine tripe and stored at 4±1°C for 28 days under aerobic and vacuum packaging conditions. The products were developed by mechanical tenderization processes by mincing and blade tenderization then cooked till the middle temperature of 83 ± 1°C was attained and stored under different packaging conditions. The evaluation of the product for microbial quality was done using the standard analytical methods at intervals of 7 days for 28 days under refrigeration conditions. The results revealed an acceptable trend which indicated good hygienic handling of products during processing. The detected bacterial counts were in the ranges specified RTE meat products by Kenya bureau of Standards (KEBS) for the 28 days storage period of vacuum packaged products. However, the microbial counts in aerobically packaged products were significantly ($p<0.05$) higher than in vacuum packed products and slight off odours and slime appeared on 28th day of storage. *Listeria monocytogens, Campylobacter, Escherichia coli and Salmonella spp* were all absent in both aerobic and vacuum packed products. The highest days means counts for *total viable counts, clostridium perfringens, staphylococcus aureus, yeast and molds, psychrophilic counts* and *lactobacillus spp* were 5.4 $\log_{10}$ cfu/g, 1.7 $\log_{10}$ cfu/g, 1.9 $\log_{10}$ cfu/g, 4.1 $\log_{10}$ cfu/g, 5.2 $\log_{10}$ cfu/g, 2.1 $\log_{10}$ cfu/g respectively in both packages. Therefore, bovine tripe rolls prepared by mechanical tenderization can best be stored for 28 days under vacuum packaging at 4±1°C and 21 days for aerobic packaging at the same temperature.

Keywords: Bovine tripe, mechanical tenderization, microbial contamination, pathogens

1. Introduction

Ready to eat (RTE) bovine tripe rolls are some of the meat products that possess huge potential for microbial spoilage. They are developed from bovine tripe which contains high quality proteins, minerals and vitamins (Seong *et al.,* 2014). All over the world, slaughter houses generate huge volumes of bovine tripe which is under-utilized due to its inherent tough nature requiring long cooking times and also highly perishable (Araba,2006). For instance, according to Ockerman and Hansen (2000), it has been estimated that 5.62kgs/cattle of bovine tripe are harvested after slaughter. The microbial quality of bovine tripe is generally poor due to readily available nutrients and unhygienic conditions during collection, handling and processing and hence can cause food borne illnesses (Abd et al., 2018).

In order to convert the highly perishable and tough bovine tripe into an attractive, more convenient and generally acceptable novel product, appropriate technologies need to be employed for value addition. Mechanical tenderizing has been used extensively to enhance the tenderness and palatability of meat and meat products (Wicklund et al., 2006). This is achieved through mincing or by use of blades and it entails penetrating and manipulating the muscles of meat or meat by-products to tenderize them (Pietrasik and Shand 2004). Mechanically tenderized meat products pose a high microbial risk to consumers since the pathogens on the surface of meat or meat by-product can be carried into the deep tissues of the cuts that were previously sterile. The newly introduced pathogens can proliferate and increase in population especially if the meat product is undercooked consequently shortening the storage life of the product (Johns et al., 2011). The complicated construction design and difficulty in disassembling the equipment used for mechanical tenderization can pose a challenge during cleaning and sanitizing which can enable the pathogens to remain within the niches of the equipment hence contaminating the product (Youssef et al., 2014).

Cooked meat products can act as the growth media for bacteria, molds and yeast some of which can be pathogenic (Jay *et al*., 2005). *Listeria monocytogens, Clostridium perfringens, Salmonella spp* and *Campylobacter* are normally of particular interest because they are indicators of food safety. They are dominant in meat products and have been associated with diseases outbreaks (Jacxsens, 2009). These microorganisms can enter the product from spices and other ingredients, equipment, improper handling and processing,

recontamination during post processing handling and storage. Proper heating of meat products during processing has been found to be effective in reduction of microbial counts of the final products (Güngör, 2010). Although cooking drastically reduces the initial population of bacteria, some vegetative cells and bacterial spores can survive. As a result, it is essential to prevent post cook contamination of the end-product by ensuring that they are stored at low enough temperature to prevent microbial growth.

Use of appropriate packaging and storage (cold treatment) condition plays a significant role in preserving the microbial quality of meat products (Lavieri and Williams, 2004). Vacuum packaging has been found to prolong the storage life of meat products by reducing undesirable bacterial growth (Strydom and Jones, 2014). Therefore, the aim of the present research was to evaluate the microbiological status of the RTE bovine tripe rolls produced by mechanical tenderization and stored under aerobic and vacuum packaging conditions at refrigeration temperatures.

2.0 Materials and methods

The study was conducted at the Department of Food Science, Nutrition and Technology, College of Agriculture and Veterinary Science, University of Nairobi. The Analytical tests were carried out in the food chemistry and food microbiology laboratories.

2.1 Sample collection and preparation

The bovine tripe was obtained from a local approved slaughterhouse (Bahati slaughterhouse, Limuru) which possesses the required authorisation from local authorities about health and hygiene practices. Other ingredients were bought from an authorized supplier in Nairobi city. Fat and other extraneous substances attached to the surface of tripe were separated using a knife followed by thorough washing to removal any adhering intestinal content and mucous lining. The tripe was then cut into (8×8cm) pieces for ease of mechanical tenderization. The already processed casings with internal diameter of 5 cm were bought from authorized processor in Nairobi city. Other ingredients were bought from an authorised supplier in Nairobi city.

2.2 Product formulation and treatments

The formulated ready to eat product consisted of the following ingredients: bovine tripe, common table salt, sodium triphosphate, $NaNO_2$, ascorbic acid, spices, and flakes of ice. A series of initial/preliminary trials were conducted in order to develop the final formula for a ready to eat bovine tripe rolls. Different additives and seasonings used in the formulations are shown in Table 1. Blade tenderization (BT) and mincing are the treatments that were used to prepare bovine tripe rolls. Bovine tripe pieces were tenderized three times using a blade tenderizer which is operated mechanically. This was followed by sectioning the tenderized pieces into even chunks of 2 cm by 2 cm and was used to prepare blade tenderized bovine tripe rolls.

Bovine tripe pieces subjected to partial freezing (-2°C) were ground twice using a meat grinding machine and a 3 mm plate and used to prepare ground/minced cooked bovine tripe rolls.

2 cm by 2cm bovine tripe chunks (Not subjected to any mechanical treatments) were used to prepare the control products.

Table 1: Additives and seasonings for a 500g product formulation

Additives	Quantity (gm.)	Seasonings	Quantity (gm.)
Common salt	8	White pepper	1
STTP	1.5	Nutmeg	0.15
Ascorbic acid	0.15	Mace ground	0.3
MSG	0.25	Coriander & Ginger	0.4
NPS	1.5		
Total	**11.4**	**Total**	**1.85**

2.3 Preparation of the product

After tenderization and mincing, bovine tripe samples (BT samples and minced samples) were weighed separately and mixed in meat mixing machine at a speed of 250rpm for 5min with sodium triphosphate and sodium chloride. After 5 minutes, ascorbic acid, additives and seasonings, $NaNO_2$ and ices flakes were incorporated into the mass and mixing proceeded for another 5 minutes in order to get a homogenous mass.

500 grams of the homogenous meat mixture was put manually into casings. Cooking of the stuffed mixture took place in hot water that had been pre-heated till the temperature at the middle reached 83 ±1°C. The temperature was retained for approximately 15 minutes. Probe thermometer was used to record the temperature. After cooking was done, the RTE bovine tripe rolls were left to cool down packed in LDPE and put in a refrigerator to chill for about 13 hours. Thereafter, the mass was cut into thin pieces using a meat slicing machine and packed under both vacuum and aerobic conditions using PET pouches. Storage of the samples was done at 4±1 °C and microbial evaluation carried out at 0, 7, 14, 21 and 28 days.

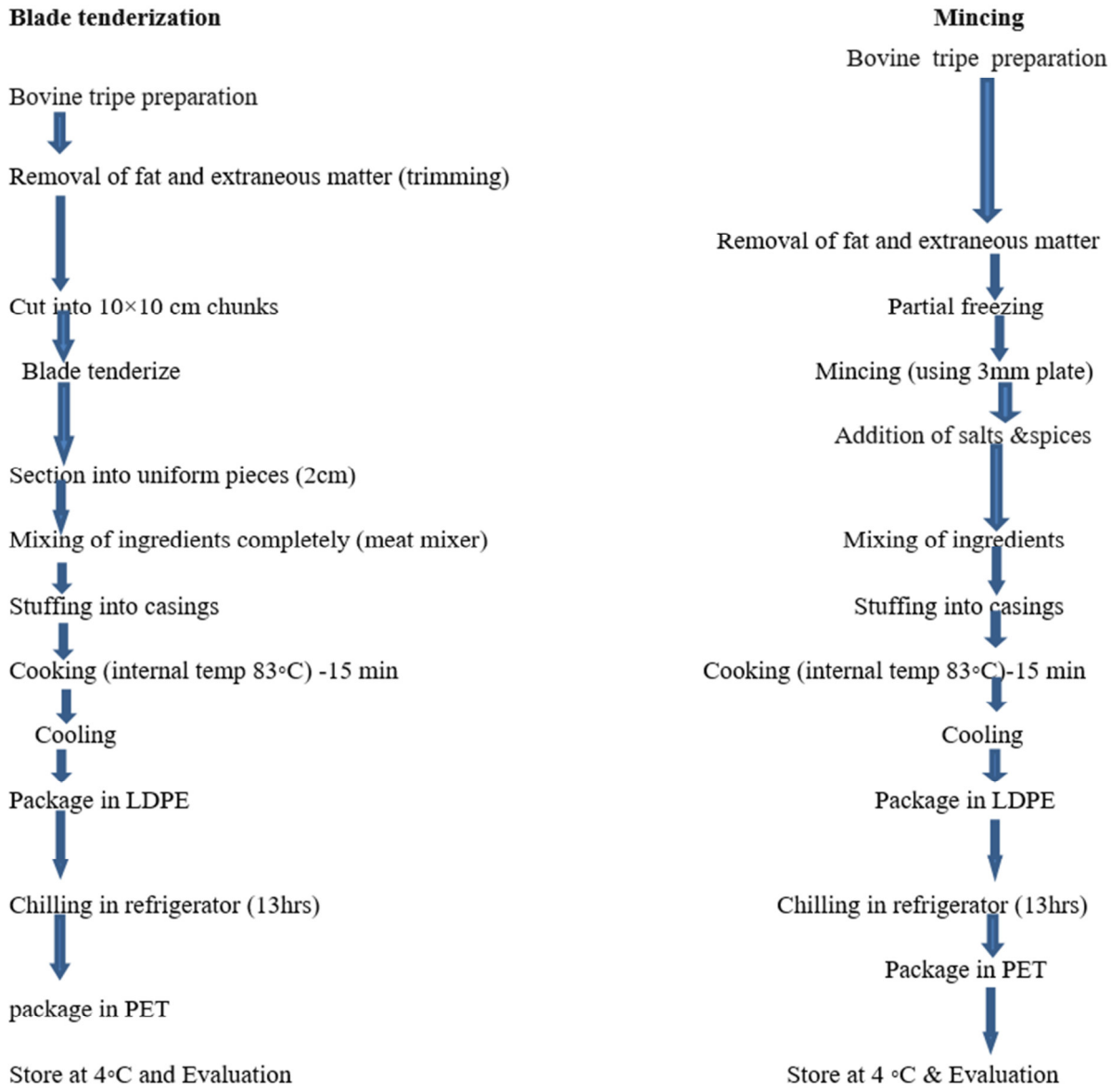

Figure 1: The RTE Bovine tripe rolls processing logical flow

2.4 Analytical methods

The RTE bovine tripe rolls were analysed for various microorganisms to evaluate the microbiological profile in order to speculate the overall keeping quality and general hygiene. The microbial tests were conducted for a period of 28 days for proper comparison with the RTE meat products in the commercial market. The objective was to get an accurate estimate of the products microbial shelf-life in relation to the conventional RTE meat products.

2.4.1 Shelf-life determination

The tests were conducted after every 7 days for 28 days throughout the refrigerated storage period. The microorganisms of interest were those relevant to safety problems of cooked meat products. *Listeria monocytogens, campylobacter, salmonella spp* and *Clostridium perfringens* were of critical significance since they act as indicators of food safety and they are dominant in meat products (Jacxsens (2009). *Staphylococcus aureus* was used to indicate the hygiene of personnel during processing while Total viable counts (TVC) were used as indicators of general microbial quality of the RTE bovine tripe rolls. Psychrophilic bacteria are nutritionally versatile and ecophysiologically resilient species and hence can grow even in refrigerated temperatures hence responsible for faster spoilage of meat products under refrigeration. *Lactobacillus spp* were used as indicators of quality deterioration since they cause souring below the casing of meat products (Jay et al., 2005). Growth of yeasts and molds forms gray slime on the surface of meat products hence discoloring it and their analysis acted as indicator of spoilage defects on the surface of the RTE tripe rolls during storage.

2.4.2 Determination of Total Viable Counts

ISO method 4833:2003 (ISO 2003) was used for enumeration of the total viable counts (TVC) Triplicate plates with plate count agar were used for enumeration. They were incubated for 72 hours at 30±1^{o}C after which bacterial counts were converted to $\log_{10}$ cfu/g of the sample.

2.4.3 Determination of *Escherichia coli*

Based on ISO method 16649-2:2001(ISO 2001), the *E. coli* will be accordingly enumerated. 8g of the sample were homogenized in 90ml of peptone water. Serial dilutions of the homogenized solution were prepared and plating done in triplicate on the selective agar media. After 48 hours of incubation at 44 ˚C, blue green colonies for *E.coli* were counted and expressed as $\log_{10}$ cfu/g of the sample.

2.4.4 Determination of Yeasts and Molds

ISO method 21527-1:2008(en) was used. Potato Dextrose agar (PDA) was used to enumerate yeasts and molds using pour plating method. Incubation of the plates was done for 5 days at 25±1 °C. The sum total of CFU of presumptive yeast and molds per gram of sample was calculated.

2.4.5 Determination of *Salmonella*

The ISO method 6579:2002 (ISO 2002) was used for enumeration of *salmonella* species. 25g of sample were homogenized with peptone water and incubated at 37±1^{o}C for 18±2 hours. The inoculums were shifted to Rappaport- Vassiliadis broth and selenite cysteine broth from the pre-enrichment broth and incubation done at 41.5±1^{o}C and 37±1^{o}C for 24 hours for selective enrichment. A loopful of the selective enrichment was streaked onto two solid selective media: Brilliant green agar (BGA) and xylose lysine desoxycholate agar (XLD). XLD agar was incubated at 37±1^{o}C and observed after 24±3 hours for typical *Salmonella* transparent red halo and a black centre.

2.4.6 Determination of *Staphylococcus aureus*

EN ISO method 6888-1:1999 (ISO 1999) was used detect and enumerate *Staphylococcus aureus.* A sterile pipette was used to transfer in triplicate the sample dilutions onto the Baird Parker agar. Incubation of the plates was then done at 35-37^{o}C for 24±2 hours, then re-incubated for further 24±2 hours. Observation was made for typical colonies appearing black or grey, shining and convex, 1-1.5mm in diameter after 24hours and 1.5-2.5mm after 48 hours of incubation, surrounded by a clear zone but partially opaque zone. The coagulase positive staphylococci were then expressed as cfu/g of sample.

2.4.7 Determination of *Clostridium perfringens*

Enumeration was done using the ISO method 7937:2004 (ISO 2004). 1ml of appropriate sample dilutions were transferred and inoculated in a sterile pipette into empty petri dishes. 10ml of the sulphite-cycloserine agar (SC) which maintained at 44-47oC in the water bath was poured into the petri dishes and mixed well with the inoculum by gently rotating each dish. After the media solidification, a 10ml over layer of the CS was added and allowed to solidify. The plates were then incubated under anaerobic conditions for 22 hours at 37^{o}C. Isolation of Clostridium perfringens was done immediately after enrichment, on iron sulphite agar at 46˚C for 18 hours. Colonies which were typically black were picked and Lactose sulphite medium test (LS) was used to perform the confirmatory test. Tubes containing lactose sulphite media were examined after 24 hours for gas production and formation of black colour. Durham tubes containing black precipitate and with gas was confirmed positive for the *Clostridium perfringens* which was expressed as cfu/g.

2.4.8 Determination of *Listeria monocytogens*

Method 11290-01:2004 (ISO 2004) was used to enumerate the organism *Listeria monocytogenes.* Fraser broth was used as a selective enrichment media and plating was done using Listeria agar and incubation was done at 37^{o}C for 24±3 hours. The confirmatory test used to confirm the *Listeria monocytogens* was carbohydrate utilization. Typical *Listeria monocytogens* colonies appear blue green with an opaque halo on the *Listeria* agar.

2.4.9 Determination of *Campylobacter*

ISO method 10272-2006 (ISO 2006) was used for enumeration. Bolton broth was used as enrichment media. A modified charcoal cefoperazonedeoxycholate agar (mCCD) was used for incubation at 41.5^{o}C for 44±4 hours. Typical colonies of *Campylobacter* are seen as greyish on mCCD agar, mostly with a metallic sheen and are flat and moist with a tendency to spread. The numbers of *campylobacter* per gram of the sample were calculated from the number of colonies per plate.

2.4.9.1 Determination of *Lactobacillus Spp*

The methods described by APHA (1992) were used for enumeration of lactobacillus spp. De Man, Rogosa and Sharpe agar (MRS agar) together with 10ml of glycerol were used to enumerate lactobacillus counts and the incubation of the plates was done for 48 hours at 35±1 °C. The numbers of *Lactobacillus* per gram of the sample were calculated from the number of colonies per plate.

2.4.9.2 Determination of *psychrophilic Counts*

The methods described by APHA (1992) were used for enumeration of Psychrophiles. A standard reference method where pour plates are incubated for 10 days at 7˚C was used for determination of pseudomonas colony counts.

2.5 Statistical analysis

After enumeration, the counts were represented as colony forming units per gram (cfu/g). Microsoft excel was used to convert the figures into logarithmic version presented as $\log_{10}$. Data was then subjected to Analysis of Variance (ANOVA) while Duncan's multiple range tests at $P \leq 0.05$ was used to compare the least significant differences of the means. Data analysis was done using Genstat version 16 for windows.

2.6 Results and Discussions

Listeria monocytogens, Campylobacter, Salmonella spp and *Clostridium perfringens* were absent in RTE bovine tripe rolls during the storage period in both vacuum and aerobic packaging conditions. Kenya Bureau of Standards legal limits require that these microorganisms be absent from the cooked meat products. According to Jacxsens (2009), *Salmonella spp, Campylobacter, Listeria monocytogens* and *Clostridium perfringens* are critical indicator microorganisms for status of food safety in the entire world. Moreover, Hsieh and Ofori (2011) reported that *Salmonella spp* and *Listeria monocytogens* caused over 1500 deaths in the USA due to contamination of food.

Most researchers have discussed several approaches of controlling microbial infestation on cooked meat products such as application of a HACCP system in the production process. However, adherence to good manufacturing practices (GMP) is a prerequisite for a successful HACCP system (Sperber *et al.*, 1998). Subjecting meat products to cold treatment especially refrigeration also works against several microorganisms including *Salmonella spp*, *Listeria monocytogens* and *Campylobacter* (Cutter and Siragusa, 1998). *Escherichia coli* have also been found in meat products made from bovine tripe by other researchers. Bachir and Mehio (2001) reported that unhygienic and improper handling of food increases the likelihood of contamination by *Escherichia coli.* Hence, the absence of *Escherichia coli* in the RTE bovine tripe rolls demonstrated proper and hygienic handling in addition with clean equipment and work area during the processing.

2.6.1 Total Viable counts (TVC)

The average day means showed a significant increase ($p<0.05$) in TVC as the storage time advanced. In spite of that, the proliferation of TVC in the middle of 14th and 21st day was not statistically significant in both aerobic and vacuum packed RTE bovine tripe rolls. The general treatment averages showed significantly more ($p<0.05$) TVC for minced bovine tripe rolls than control (non-tenderized) bovine tripe rolls. This is attributable to the higher surface area of the minced products hence easily penetrated by microorganisms. However, the total treatment means for minced and blade tenderized products in both packaging conditions did not differ significantly ($p<0.05$). The TVC during the entire storage period were well within the Kenyan safe limits of 6.0 $\log_{10}$ cfu/g for meat products as stipulated by Kenya bureau of standards (KEBS). Generally, vacuum packaged products had lower TVC than aerobically packed products. This is due to inhibitory effects on growth of microorganisms by vacuum packaging (Huffman et al., 1975 and Maqsood et al., 2016). Similar findings of increase in TVC as storage period advanced were reported by Devatkal and Mendiratta (2001) when they studied storage of pork rolls that had been restructured. The above results are also in line with the findings of Holy and Holzapet (1988) who found that the storage life of refrigerated and vacuum packaged comminted beef was better than for aerobically packaged ones.

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Table 2: Total viable counts profile ($\log_{10}$ cfu/g) of RTE Bovine tripe rolls under different packaging conditions during refrigerated storage

Storage condition	Treatment*	Storage period (days)					Treatment means ± SE
		0	7	14	21	28	
Vacuum	Control	2.1 ± 0.04^{a}	3.4 ± 0.25^{b}	4.1 ± 0.13^{c}	4.5 ± 0.21^{c}	4.6 ± 0.22^{c}	3.7 ± 0.17^{A}
	Minced	2.2 ± 0.12^{a}	3.8 ± 0.09^{b}	4.5 ± 0.25^{c}	4.9 ± 0.24^{c}	5.1 ± 0.31^{d}	4.1 ± 0.20^{B}
	BT tripe	2.1 ± 0.06^{a}	3.6 ± 0.14^{b}	4.4 ± 0.13^{c}	4.8 ± 0.48^{c}	4.9 ± 0.24^{c}	4.0 ± 0.21^{B}
Aerobic	Control	2.2 ± 0.12^{a}	3.5 ± 0.14^{b}	4.5 ± 0.23^{c}	4.6 ± 0.13^{c}	5.7 ± 0.06^{d}	4.1 ± 0.14^{A}
	Minced	2.3 ± 0.05^{a}	3.9 ± 0.05^{b}	4.8 ± 0.41^{c}	4.9 ± 0.03^{c}	6.0 ± 0.08^{e}	4.4 ± 0.12^{B}
	BT tripe	2.1 ± 0.07^{a}	3.8 ± 0.06^{b}	4.6 ± 0.02^{c}	4.9 ± 0.11^{c}	5.9 ± 0.15^{e}	4.3 ± 0.08^{B}
Average		2.2 ± 0.08^{a}	3.7 ± 0.12^{b}	4.5 ± 0.20^{c}	4.8 ± 0.20^{d}	5.4 ± 0.18^{e}	

***Values with different letters (lowercase along the row and upper case along the column) were significantly different at p<0.05. BT-tripe- Blade tenderized tripe rolls; Minced- Minced bovine tripe rolls; Control- Non tenderized tripe bovine tripe rolls.**

2.6.2 Staphylococcus aureus

The average counts for *Staphylococcus aureus* were between 1.2 and 1.9 $\log_{10}$ cfu/g in both storage conditions (Table 3). The counts increased progressively as storage time advanced in the three treatments under both packaging conditions but the counts were slightly higher in aerobic than vacuum packed products. The results are in accordance with Brenesselova et al., (2015) who noticed higher staphylococcal counts in aerobically packed ostrich meat in comparison to vacuum packaged one. Staphylococcus species are capable of growing both aerobically and anaerobically since they are facultative anaerobic organisms hence they multiplied during storage leading to increase in their population. However, the numbers fell within the KEBS allowable legal limit of $\log_{10}$ 2.0 colony forming unit per gram. Minced and blade tenderized tripe rolls treatment averages were non-significant ($p<0.05$) after 28 days of storage in both aerobic and vacuum packaging conditions. *Staphylococcus aureus* indicates the efficacy of proper handling food products during processing. Bachir and Mehio (2001) observed that improperly handled food introduces pathogens to the final product. Staphylococci are all over the environment of the humans and those present in the nose often contaminates the hands, face and fingers as food handlers' involuntary moves their hands hence increasing chances of cross-contamination (Lues and Van, 2007). This accounts for the presence of Staphylococcus *aureus* in the Bovine tripe rolls. The results for this study were in line with the findings of Anna et al., (2012) who observed continuous rise of staphylococcal counts in meat rolls stored under vacuum and refrigerated conditions.

Table 3: *Staphylococcus aureus profile* ($\log_{10}$ cfu/g) of RTE Bovine tripe rolls under different packaging conditions during refrigerated storage.

Storage condition	Treatment*	Storage period (days)					Treatment means ± SE
		0	7	14	21	28	
Vacuum	Control	1.1 ± 0.07^{a}	1.2 ± 0.04^{a}	1.4 ± 0.03^{a}	1.5 ± 0.07^{a}	1.8 ± 0.06^{b}	1.4 ± 0.05^{A}
	Minced	1.3 ± 0.06^{a}	1.4 ± 0.02^{a}	1.6 ± 0.09^{a}	1.7 ± 0.02^{b}	1.7 ± 0.03^{d}	1.5 ± 0.04^{B}
	BT tripe	1.2 ± 0.08^{a}	1.3 ± 0.03^{a}	1.5 ± 0.05^{a}	1.6 ± 0.08^{a}	1.9 ± 0.07^{c}	1.5 ± 0.06^{B}
Aerobic	Control	1.2 ± 0.12^{a}	1.3 ± 0.03^{a}	1.5 ± 0.13^{a}	1.7 ± 0.11^{b}	1.8 ± 0.09^{d}	1.5 ± 0.10^{A}
	Minced	1.3 ± 0.05^{a}	1.5 ± 0.02^{a}	1.7 ± 0.28^{b}	1.8 ± 0.02^{b}	1.9 ± 0.04^{c}	1.6 ± 0.08^{B}
	BT tripe	1.2 ± 0.07^{a}	1.4 ± 0.05^{a}	1.6 ± 0.01^{a}	1.9 ± 0.04^{c}	2.0 ± 0.16^{c}	1.6 ± 0.07^{B}
Average		1.2 ± 0.08^{a}	1.4 ± 0.03^{b}	1.6 ± 0.10^{c}	1.7 ± 0.06^{c}	1.9 ± 0.08^{d}	

***Values with different letters (lowercase along the row and upper case along the column) were significantly different at p<0.05. BT-tripe- Blade tenderized tripe rolls; Minced- Minced bovine tripe rolls; Control- Non tenderized tripe bovine tripe rolls**

2.6.3 Clostridium Perfringens

The overall day means for *Clostridium perfringens* counts were between 1.7 l - 1.1 $\log_{10}$ cfu/g (Table 3). The decreasing trend observed in both aerobic and anaerobic packages throughout the storage period is attributable to the low storage temperatures. These results corroborates with the findings of Juneja et al., (1993) who reported a progressive decrease in *Clostridium perfringens* counts in vacuum and aerobically packed cooked beef during refrigerated storage. The highest counts were 1.8$\log_{10}$ cfu/g on day 0 for minced bovine tripe rolls while the lowest counts were on day 28 at 1.0 $\log_{10}$ cfu/g for blade tenderized and control products. The counts were way below the set lethal limits of 4.0 $\log_{10}$ cfu/g stipulated by KEBS. The overall treatment means showed no significant ($p<0.05$) difference among all the products in both aerobic and vacuum packaging. *Clostridium perfringens* are important indicators of food safety and they are linked to bans imposed on meat products by importing countries (Heinitz *et al*., 2000). The importance of *Clostridium perfringens* is the increasing reports that some strains can proliferate at refrigeration temperatures previously thought to inhibit growth of pathogenic microorganisms (Johnson, 1990).

Table 3: *Clostridium Perfringens* profile ($\log_{10}$ cfu/g) of RTE Bovine tripe rolls under different packaging conditions during refrigerated storage

Storage condition	Treatment*	Storage period (days)					Treatment means ± SE
		0	7	14	21	28	
Vacuum	Control	1.6±0.02^{a}	1.4±0.10^{a}	1.3±0.08^{a}	1.2±0.01^{ab}	1.2±0.11^{ab}	1.3±0.11^{A}
	Minced	1.8±0.01^{a}	1.4±0.02^{a}	1.3±0.06^{a}	1.1±0.02^{b}	1.1±0.03^{b}	1.3±0.04^{A}
	BT tripe	1.7±0.07^{a}	1.5±0.02^{a}	1.5±0.05^{a}	1.2±0.06^{ab}	1.2±0.02^{ab}	1.4±0.05^{A}
Aerobic	Control	1.7±0.01^{a}	1.3±0.12^{a}	1.2±0.02^{ab}	1.1±0.11^{b}	1.0±0.01^{b}	1.3±0.10^{A}
	Minced	1.8±0.02^{a}	1.3±0.01^{a}	1.3±0.04^{a}	1.2±0.02^{b}	1.1±0.02^{b}	1.3±0.08^{A}
	BT tripe	1.5±0.01^{a}	1.4±0.03^{a}	1.2±0.03^{ab}	1.1±0.04^{b}	1.0±0.02^{b}	1.2±0.07^{A}
Average		1.7±0.02^{a}	1.4±0.05^{b}	1.3±0.05^{b}	1.2±0.04^{b}	1.1±0.04^{b}	

***Values with different letters (lowercase along the row and upper case along the column) were significantly different at p<0.05. BT-tripe- Blade tenderized tripe rolls; Minced- Minced bovine tripe rolls; Control- Non tenderized tripe bovine tripe rolls**

2.6.4 Yeasts and molds

Yeast and mold counts were not detected on day 0 in both packaging conditions. This is attributable to environmental stress especially cooking that subjected the cells to metabolic injury hence they were unable to form colonies. Nonetheless, as the storage period advanced, the injured cells got repaired and were able to form colonies on 7th day (Anna et al., 2012). Mold and yeast counts progressively increased with storage period in both packaging conditions. The vacuum packed products had significantly lower ($p<0.05$) yeast and mould counts compared to aerobically packed products during the entire storage period. This could be attributed to higher amount of oxygen in aerobic packages that facilitated faster proliferation of yeast and molds (Malik and Sharma, 2014).The ability of vacuum packaging to retard the multiplication of molds and yeast during storage was also reported by Rao et al., (2005). Minced and blade tenderized products treatment averages showed a significantly higher ($p<0.05$) molds and yeast counts compared to control. However, the difference between minced and blade tenderized rolls in both aerobic and vacuum conditions was non-significant ($p<0.05$). The above results are in agreement with Malik and Sharma (2014) who reported significantly higher yeasts and mold counts in aerobically packaged RTE meat products compared to vacuum packaged ones.

Table 4: Yeasts and molds profile ($\log_{10}$ cfu/g) of RTE Bovine tripe rolls under different packaging conditions at refrigerated storage

Storage condition	Treatment*	Storage period (days)					Treatment means ± SE
		0	7	14	21	28	
Vacuum	Control	ND	2.3±0.28^{a}	3.2±0.02^{a}	3.5±0.25^{a}	3.5±0.12^{a}	3.1±0.11^{A}
	Minced	ND	2.6±0.15^{a}	3.3±0.22^{a}	3.4±0.17^{a}	3.9±0.14^{bc}	3.3±0.04^{B}
	BT tripe	ND	2.6±0.11^{a}	3.6±0.23^{b}	3.7±0.09^{b}	4.1±0.18^{c}	3.5±0.05^{B}
Aerobic	Control	ND	2.5±0.14^{a}	3.5±0.21^{a}	3.9±0.26^{bc}	3.9±0.02^{bc}	3.5±0.10^{A}
	Minced	ND	2.8±0.18^{a}	3.7±0.06^{b}	4.2±0.08^{c}	4.5±0.12^{d}	3.8±0.11^{B}
	BT tripe	ND	2.9±0.12^{a}	3.8±0.10^{b}	4.3±0.06^{c}	4.4±0.28^{d}	3.9±0.14^{B}
Average			2.6±0.16^{a}	3.5±0.14^{b}	3.8±0.15^{c}	4.1±0.14^{d}	

***Values with different letters (lowercase along the row and upper case along the column) were significantly different at p<0.05. BT-tripe- Blade tenderized tripe rolls; Minced- Minced bovine tripe rolls; Control- Non tenderized tripe bovine tripe rolls; ND- Not Detected**

2.6.5 Lactobacillus spp

The average day's means showed significant increase in *Lactobacillus spp* counts as the storage period advanced in both packaging conditions. However, the increase was not significant ($p<0.05$) on day 0 and day 7 in all the

products. Similar trend was reported by Masqood et al., (2016) who observed progressive increase in lactobacillus counts during refrigerated storage of camel meat. Jones et al., (1988) also reported continuous increase of *lactobacillus spp* counts of restructured meat products during storage at low temperature under vacuum packaging. Average treatment means showed non-significant difference between the control, minced and blade tenderized bovine tripe rolls. The average treatment means indicated significantly higher ($p<0.05$) counts in vacuum packaged products compared to aerobically packaged ones. These results are in accordance with the findings of Doulgeraki et al., (2012) who reported high counts of *lactobacillus spp* in vacuum packed meat and meat products stored at 4°C during storage. The lactobacillus counts were between the specified range for meat products despite the increase in their counts during the refrigerated storage (Jay et al., 2005).

Table 4: *Lactobacillus spp* profile (log_{10} cfu/g) of RTE Bovine tripe rolls under different packaging conditions during refrigerated storage

Storage condition	Treatment*	Storage period (days)					Treatment means ± SE
		0	7	14	21	28	
Vacuum	Control	1.3±0.18^{a}	1.4±0.12^{a}	1.8±0.06^{b}	2.3±0.11^{c}	2.3±0.21^{c}	1.8±0.14^{A}
	Minced	1.5±0.11^{a}	1.5±0.06^{a}	1.9±0.16^{b}	2.1±0.12^{bc}	2.4±0.15^{c}	1.9±0.12^{A}
	BT tripe	1.4±0.06^{a}	1.5±0.13^{a}	1.8±0.08^{b}	2.3±0.10^{c}	2.4±0.11^{c}	1.9±0.11^{A}
Aerobic	Control	1.4±0.02^{a}	1.4±0.26^{a}	1.5±0.08^{a}	1.5±0.12^{a}	1.8±0.16^{b}	1.5±0.13^{B}
	Minced	1.2±0.18^{a}	1.3±0.10^{a}	1.4±0.11^{a}	1.5±0.09^{a}	1.9±0.05^{b}	1.5±0.11^{B}
	BT tripe	1.3±0.06^{a}	1.4±0.11^{a}	1.5±0.11^{a}	1.5±0.06^{a}	1.8±0.04^{b}	1.5±0.08^{B}
Average		1.4±0.10^{a}	1.4±0.13^{a}	1.7±0.10^{b}	1.9±0.10^{c}	2.1±0.11^{d}	

***Values with different letters (lowercase along the row and upper case along the column) were significantly different at p<0.05. BT-tripe- Blade tenderized tripe rolls; Minced- Minced bovine tripe rolls; Control- Non tenderized tripe bovine tripe rolls**

2.6.6 Psychrophilic counts

There were no colonies on the initial day of storage in minced, blade tenderized as well as control (non-tenderized) products in both aerobic and vacuum packaging conditions. This is attributable to retardation of log phase due to abrupt adjustment of the physical environment which caused reduction in metabolic rate of the Psychrophiles (Gupta and Sharma, 2017). As the storage period progressed, the injured cells regenerated leading to formation of colonies on the 7th day of storage. Gadekar *et al.,* (2014) observed psychrophilic counts form 15th day going forward during the storage of restructured meat products from goat. From day 7, psychrophilic counts increased significantly ($p<0.05$) in all the products in both packages as the storage period advanced. However, the counts were higher in aerobically packaged products compared to vacuum packaged ones throughout the storage period. Anna et al., (2012) deduced that raised levels of CO_2 as a result of vacuum packaging retarded the proliferation of psychrophilic bacteria hence the lower counts. The results of this study are in line with the findings of Maqsood et al., (2016) who found that the psychrophilic counts of vacuum packaged camel meat samples were lower than counts for aerobically packed ones during the refrigerated storage. The overall treatment means in respective packaging conditions did not did not differ significantly. The appearance of visible slime and off odors in aerobically packaged products on the 28th day showed a marginal spoilage stage which is a major food safety concern. Other researchers have reported that vacuum packaging is more efficient in reducing psychrophilic counts of meat products compared to aerobic packaging (Bingol & Ergun, 2011; Fernandez-Lopez et al., 2008; Lorenzo & Gomez, 2012).

***Psychrophilic bacterial counts* profile (log_{10} cfu/g) of RTE Bovine tripe rolls under different packaging conditions during refrigerated storage**

Storage condition	Treatment*	Storage period (days)					Treatment means ± SE
		0	7	14	21	28	
Vacuum	Control	ND	2.5±0.18^{a}	3.1±0.05^{b}	4.3±0.18^{c}	4.4±0.28^{c}	3.6±0.14^{A}
	Minced	ND	2.6±0.22^{a}	3.7±0.21^{b}	4.2±0.31^{b}	4.6±0.23^{c}	3.8±0.24^{A}
	BT tripe	ND	2.3±0.15^{a}	3.6±0.32^{b}	4.4±0.33^{c}	4.5±0.42^{d}	3.7±0.31^{A}
Aerobic	Control	ND	2.8±0.06^{a}	3.3±0.26^{b}	4.8±0.11^{c}	5.6±0.16^{e}	4.1±0.15^{B}
	Minced	ND	2.5±0.08^{a}	3.8±0.02^{b}	4.9±0.27^{c}	5.9±0.05^{e}	4.2±0.11^{B}
	BT tripe	ND	2.4±0.05^{a}	3.7±0.12^{b}	4.6±0.07^{c}	6.1±0.04^{e}	4.2±0.07^{B}
Average			2.5±0.12^{a}	3.5±0.16^{b}	4.5±0.21^{c}	5.2±0.20^{d}	

***Values with different letters (lowercase along the row and upper case along the column) were significantly different at p<0.05. BT-tripe- Blade tenderized tripe rolls; Minced- Minced bovine tripe rolls; Control- Non tenderized tripe bovine tripe rolls. ND- Not Detected.**

2.7 Conclusion

This study revealed that it is practically possible to obtain tenderized meat product with a good microbiological

quality by use of mechanical tenderization methods. Proper and hygienic handling practices during processing are paramount to ensure an acceptable general hygiene. The results of this study showed that vacuum packaging is more effective in slowing down microbial growth and maintaining the quality of meat products for longer period compared to aerobic packaging. The study also indicates that mechanically tenderized RTE bovine tripe rolls can be stored at 4±1 °C for 28 days under vacuum packaging and 21days under aerobic packaging without significantly affecting the microbial quality.

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