Assessment of microbial quality of fresh beef tongue meat sold in various markets in Khartoum, Sudan

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Abstract

Microbial quality assessment of beef tongue meat products sold within Khartoum town was conducted between February-April 2017. A total of fifteen (15) samples were purchased from five different spots and analyzed microbiologically using the pour plate method. The total aerobic plate count on nutrient agar ranged from $1.4x10^4$ cfu/ml to $2.95x10^5$ cfu/ml. The coliform count using most probable number technique (MPN) ranged from 3 cfu/g-240 cfu/g. Total fungal count (yeast and mold) on Sabaroud dextrose agar with *Chloramphenicol* (as control) ranged from $1x10^3$ cfu/ml- $8x10^3$ cfu/ml. The bacteria isolated include *Staphylococcus aureus, Escherichia coli, Shigella spp*, and *Salmonella* spp. The percentage of occurrence of bacterial isolates was highest in *Staphylococcus aureus* (43.5%), *Shigella spp* (21.7%), *Salmonella spp* (21.7%) and *Escherichia coli* (13.0%) recorded the lowest. It is concluded that the occurrence of such organisms indicates contamination of the tongue meat samples. Hence, proper care in the course of preparation and handling of tongue meat needs to be established. Educating the meat handlers on the issue of food safety and public health will reduce the rate of contamination of the tongue meat.

Key words Aerobic plate count; Coliforms count; Microbial quality assessment; Beef tongue meat; Khartoum

1. Introduction

The wide diversities in Sudanese cultural, economic, tribal, ethnic, geographical and religious status ought to have vast and serious hygienic implications on food. Food cultivation, transport, processing, retailing, preservation and handling when coupled with those diversities could aggravate the hygienic situation of food (Basset, 1981; Frazier, 1992).

Meat and meat products form an important segment of the human diet because they provide essential nutrients which cannot be easily obtained through vegetables and their derived products (Byers, 2002). They provide a means for reducing malnutrition and increasing household food and food security (Chikwanha et al., 2017). Over the last 20 years, the demand for meat and meat products has increased in many parts of the world (including Africa, Asia, Europe and United States of America) and this has led to rapid surge in livestock production for sustainable food security (Sans and Combris, 2015). The process of converting livestock to meat in abattoirs usually generates a lot of by-products which can be further utilized by humans as food or reprocessed as secondary by-products for both agricultural and industrial uses (Liu, 2002). The yield of these by-products has been reported to account for about 10% to 15% of the value of the live animal in developed countries, although animal by-products account for about two-third of the animal after slaughter (Irshad and Sharma, 2015). Basically, animal

by-products include all parts of a live animal that are not part of the dressed carcass such as liver, heart, rumen contents, kidney, blood, fats, spleen and tongue.

Research on tongues has been limited. Frentz (1978) described the preparation of cured smoked beef products while Miller (1988) evaluated the physical and sensory properties of jerky processed from beef tongue. Some studies have been conducted on unprocessed tongue and similar types of meat; for example, Bersani et al., (1984) investigated *Psychrotrophic enterobacteriacea* occurring in refrigerated tongues, and the effects of lactic acid decontamination and vacuum packaging on the quality of veal tongues were studied by Visser et al., (1988). The most recent report relating to tongue was concerned with microbiological contamination in beef tissues conducted by Delmore et al. (2000). Their results showed that acetic acid (2%) spraying, lactic acid (2%) immersion, and hot water (78–80° C) spraying for 10 s were among the most effective treatments for reducing Aerobic Plate Counts (APCs), total coliform counts, and *E. coli* counts on a variety of meats including tongue.

Sudan exports livestock carcasses to many regional countries without offal meats. It appears that there is a surplus tongue meat in the local market. It is thought that it is feasible to incorporate tongue meat into different processed meat products. The present study was planned to achieve the following objectives;

- To assess the microbial quality of fresh beef tongue sold in Khartoum markets.
- To determine the microbial (*Staphylococcus, Escherichia coli, Salmonella,* total viable count and total yeast and mold) Load of fresh beef tongue sold in Khartoum markets.

2. Materials and Methods

2.1 Sample collection

A total of fifteen samples of Tongue meat were purchased from five l,ocal markets, three (3) samples each from five (5) different spots (Khartoum, Bahri, Omdurman, Elkadaro, Elhaj yosof) within Khartoum town. Purchased samples were collected and labeled as A, B, C, D and E and then hygienically transferred in ice boxes. The samples were then transported to the Microbiological Laboratory of Khartoum University. Microbiological analyses were processed immediately to avoid further contamination.

A1, A2. A3= Samples from Khartoum local market
B1 B2 , B3= Samples from Bahri local market
C1. C2, C3= Samples from Omdurman local market
D1. D2. D3= Samples from Alkadaro local market
E1. E2. E3 = Samples from El-Hajyosof local market

2.2. Sample preparation

One gram (1) of the Tongue meat sample was weighed, cut into pieces using a sterile knife and then aseptically introduced into 9ml of distilled water; it was properly shaken and was used as stock. Several dilutions were achieved up to 4 fold (10^{-4}) for each prepared sample using 1ml from stock homogenate and 9ml of sterile distilled water for serial dilution. This was carried out in order to obtain discrete colony (Moshood et al., 2012).

2.3. Bacterial isolation and determination of total viable counts

2.3.1. Aerobic plate count

The total aerobic plate count is useful for indicating the overall microbiological quality of a product and thus, is useful for indicating potential spoilage in perishable food products. The aerobic plate count is also useful for indicating the sanitary conditions under which the food was produced and**/**or processed. Andrews, (1992)**.** 225 ml of Butterfield's phosphate buffer were added to a blender jar containing 25 g analytical unit of product and blended for 2 minutes at 10,000 - 12,000 rpm (round per minute). This resulted in a dilution of 10-**1.**

Using separate sterile pipets, decimal dilutions of 10-**2,** 10-**3,** 10-**4,** and others were prepare, as appropriate of food homogenate, transferring 10 ml of previous dilutions to 90 ml of diluent. Shaking all dilutions 25 times within 7 seconds. 1 ml of each dilution was Pipeted into separate, duplicate and appropriately marked

Petri plates. 20 ml of plate count agar (cooled to 44–46°C) were added to each plate within 15 min of original dilution. Sample dilutions and agar medium were thoroughly and uniformly mixed. Agar was allowed to solidify; Petri plates were inverted, and incubated promptly for 48 + 2 hours at 350C. After incubation, duplicate plates having 25 - 250 colonies range were counted. When only one dilution was in appropriate range, average count per g for dilution was computed and reported as aerobic plate count per g. when 2 dilutions were in appropriate range, average count dilution was determined before averaging tow dilution counts to obtain aerobic plate count per g. count were rounded to 2 significant figures only at time of conversion to aerobic plate counts. (Andrews, 1992).

2.3.2. ***Isolation and enumeration of Staph. aureus***

For each dilution to be plated one ml sample suspension was aseptically transferred to three plates of Baird-Parker agar medium, distributing 1 ml of inoculum equitably to three plates, Inoculums were spreaded over surface of the agar plate, using sterile bent glass streaking rod. Plates were retained in upright position until inoculum was absorbed by agar medium, (about 10 minutes on the dried plates). Plates were inverted and incubated for 48 hours at 350C, plates containing 20-200 colonies were selected, unless only plates at lower dilutions (> 200 Colonies) had colonies with typical appearance of *Staph. Aureus.*
Colonies of *Staph. aureus* were typically circular , smooth convex,
moist, 2-3 mm in diameter on uncrowded plates, gray to jet-black
frequently with light-coloured (off-white) margin, surrounded by opaque
zone and frequently with an outer clear zone;

Colonies were counted and recorded. If several types of colonies that appeared to be *Staph. aureus* were present; the number of colonies of each type were counted. Plates of the lowest dilution plated, which contain < 20 colonies were used. If plates containing > 200 colonies of typical *Staph. aureus* appearance and plates of higher dilution had no typical colonies; these plates were used to enumerate *Staph. aureus.*

More than one colony of each type counted was selected and tested for Coagulase production**.** Numbers of colonies on set of triplicate plates represented by colonies giving positive coagulase test were added and multiplied by sample dilution factor. (Andrews, 1992).

2.3.3. Salmonella Spp.

2.3.3.1. Sampling and laboratory examination

Cotton wool medical swabs from carcass sites –or direct sampling from the processed meat products_ were used for sampling. These were taken directly to laboratory and within an hour each was transferred into 10 ml enrichment medium (Selenite broth base– (Oxoid cm 395) with added Sodium Biselenite– (Oxoid L 121), (Hobbs and Allison, 1945). The enrichment media were incubated at 420C for 24 hours and then subcultured onto Brillient Green Agar plates (Oxoid cm 329) and incubated at 370C for 24 hours .Subculturing was repeated after 24 hours for all plates with no growth or red colonies (Linton, et al, 1981). Red colonies were picked and identified by serological and biochemical tests.

2.3.3.2. Serological Identification (Edward and Ewing, 1972**)**

Red colonies from Brilliant Green agar plates were either purified by further subculturing on MacConkey agar plates (Oxoid cm 76) incubated at 37°C for 24 hours or were tested directly by slide agglutination against *Salmonlla* Polyvalent O, Polyvalent H,(specific and non-specific) sera. If results were positive further slide Agglutination tests were used with single factor sera to determine O and H antigens. (Wellcome salmonella agglutination sera were used).

2.3.3.3. Biochemical Identification

Two media were used for these tests.
i) - Kohn Two- tube Medium (Oxoid cm 179).
ii) - Triple Sugar Iron Agar (Oxoid cm 277).

Red colonies from Brillient green agar plates were inoculated for confirmation onto these media and incubated at 370c for 24 hours.

2.3.4. Test for coliforms using three tubes (most probable number) techniques

Aseptically, 10g of the tongue meat sample was weighed and transferred into 90ml of sterile lactose broth which served as stock. Decimal dilution of 1:10-1 to 1:10-3 was prepared by adding 1ml of the previous dilution (stock) to 9ml of the sterile lactose broth. Three (3) replicate tubes containing durham tubes and lactose broth per dilution were prepared with 1ml of the previously prepared 1:10, 1:100, and 1:1000 dilutions. The tubes were then incubated for 24 hours at 35 °C and were observed for gas production. Negative tubes were incubated for an additional 24hours. All tubes showing gas within 48±2hours were recorded. The number counted was referred to as Most Probable Number (MPN) for the three tubes dilution.

2.3.4.1. Bacteria identification

The distinct colonies that develop in the pure culture plate were observed for the morphological and cultural characteristics including the nature of margin, elevation, shape, colour and transparency and Gram staining [10-12] and set of Biochemical Characterization i.e. indole test, Methyl-Red test, Vogues-Proskauer test and Citrate utilization test, catalase test, coagulase test and oxidase test by standard method given by Sherman (2005) and Holt et al., (1994).

2.3.5. Total moulds

Moulds were enumerated by surface plating on Malt Extract Agar (Oxoid) with 0.01% chloramphenicol as bacterial inhibitor and incubated aerobically at 25ºC for 2-3 days (Harrigan and MacCance, 1976).

2.3.6. Total Yeasts

The enumeration of yeast was done in Yeast Extract Agar; plates were incubated at 30°C for 3 days Harrigan (1998).

2.3.7. Staphylococcus aureus

Staphylococcus aureus was performed on Baird-Parker Agar (Oxoid). The plates were incubated at 37ºC for 48 h. (Harrigan and MacCance, 1976)

3. Results and Discussion

The total aerobic plate count, total fungal count, and coliform count (MPN) of the samples was presented in Table 1. The result showed that sample A1 had the highest total aerobic plate count with $2.95x10^5$, while the least total aerobic plate counts were recorded in sample E2 with $1.4x10^4$. The result also indicated that sample A2 had the highest total fungal counts of $8x10^3$ while all samples in C recorded the least total fungal counts of $1x10^3$. Additionally, for coliforms count, sample A1 was the highest with 240 coliform counts and sample E2 recorded the least coliforms counts of 3. However, the result showed that the samples were within the acceptable WHO standard limit. The morphological, microscopic description and biochemical characterization of four (5) different bacteria isolated from the fifteen different Tongue samples was presented in Table 2. The bacteria identified were *Salmonella spp*, *Shigella spp*, *Staphylococcus aureus* and *Escherichia coli*. The morphological and microscopic description of two different fungal species which were isolated from the samples was presented in Table 3. The species identified were *Aspergillus niger*, and *Penicillium spp*. The distribution of the isolated bacteria was presented in Table 4. The result showed that a total number of Twenty three (23) bacteria were isolated from the tongue samples, *Staphylococcus aureus* was the most common bacteria isolated with 10 (33.3%), followed by *Shigella* 5 (21.7%) and *Salmonella spp* with 5 (21.7%) while *Escherichia coli* had 3 (13.0%).

This study shows that the Tongue meat analyzed contained a high number of microorganisms, this could be due to possible source of contamination such as, through slaughtering of sick animals, washing the meat with dirty water, handling by butchers, contamination by flies processing close to a refuse dump environment, spices, transportation and use of contaminated equipments such as knife and other utensils Igyor and Uma, (2005), Gilbert and Harrison, (2001). There are lots of factors affecting the growth of microorganisms on meats, these factors include temperature, pH, water availability, presence of nutrients, moisture, acidity (intrinsic factors),

Table 1: Result of total aerobic plate count, total fungal count, and coliform count (MPN) of the beef tongue meat samples analyzed. TPC: Total Aerobic Plate Count; TFC: Total Fungal Count.

S/N	Sample	TPC (CFU/ml)	TFC (CFU/ml)	Coliform count (MPN/g)	Conformity with Standard Limits
1	A1	$2.95X10^5$	$5X10^3$	240	Pass
2	A2	$9.2X10^4$	$8X10^3$	150	Pass
3	A3	$1.19X10^5$	$5X10^3$	240	Pass
4	B1	$8.7X10^4$	$2X10^3$	43	Pass
5	B2	$7.7X10^4$	$1X10^3$	21	Pass
6	B3	$6.2X10^4$	$1X10^3$	23	Pass
7	C1	$1.10X10^5$	$1X10^3$	21	Pass
8	C2	$7.8X10^4$	$1X10^3$	14	Pass
9	C3	$8.6X10^4$	$2X10^3$	14	Pass
10	D1	$1.98X10^5$	$2X10^3$	210	Pass
11	D2	$1.63X10^5$	$1X10^3$	210	Pass
12	D3	$6.5X10^4$	$4X10^3$	150	Pass

Table 2: Morphological and biochemical properties of bacteria isolated from Tongue meat samples collected from Khartoum Town. Key: 1= Catalase, 2 =Coagulase, 3=Citrate, 4=Indole, 5 =Methyl red, 6= Voges- proskeur.

Isolates	Colonial Morphology	Gram Reaction	1 2 3 4 5 6	Organism
A	Small circular colonies with black center	Gram negative rods	+ - - - + -	*Salmonella spp*
B	Small circular colonies without black center	Gram negative rods	+ - - - + -	*Shigella spp*
C	Golden yellow colonies	Gram positive cocci	+ + + - + +	*S. aureus*
D	Green metallic sheen	Gram negative rods	+ - - + - -	*E. coli*

Table 3: Morphological and microscopic description of fungal isolates.

Isolates	Colonial Morphology	Microscopy
A	Dark brown to black pigmentation	Numerous mycelia conidiophores are black spherical to oval, produced in chain
B	Colony has shades of green with white	Single-celled conidia produced with brush-like spore-bearing structures

gaseous requirement and atmosphere of storage (extrinsic factors) as stated by Nester et al.,(2001). The variations in aerobic plate counts, fungal counts as well as in the coliform counts recorded in this study could be due to the materials and different treatment/processes used by the producers and sellers of the tongue meat. This observation is in line with the findings of Igene et al., (2009) which stated that the quality of tongue produced by the processors varies from one producer to another due to lack of standard method of preparation that would ensure consistent product quality. The aerobic plate counts ranged from $1.4x10^4$ to $2.95x10^5$ colony forming units

(cfu) per ml. Inyang et al., (2005) recorded comparably similar values for total plate counts in tongue samples in the order of 10^5 and 10^6 and stated that the values were within satisfactory limits according to the International Commission of Microbiological Standards of Foods (Onourah et al., 2005).

In this study, four different bacteria were isolated and identified which are *Staphylococcus aureus*, *Salmonella spp*, *Shigella spp* and *Escherichia coli* and similar findings of some of these bacteria have been earlier reported by several workers (Uzeh et al., 2006; Falegan et al., 2017). Bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp*, and *Shigella spp* observed in this study are of public health importance as they have been found

Table 4: Occurrence of bacterial isolates in tongue samples from Khartoum Town. -=Absence of isolates, +=Presence of isolates

S/N	Samples	*S. aureus*	*E. coli*	*Shigella*	*Salmonella*
1	A1	-	+	+	-
2	A2	+	+	-	+
3	A3	+	-	+	-
4	B1	-	-	-	-
5	B2	+	-	-	-
6	B3	+	-	-	-
7	C1	+	-	+	+
8	C2	-	-	-	-
9	C3	-	-	-	-
10	D1	+	+	+	+
11	D2	+	-	-	-
12	D3	+	-	-	+
13	E1	-	-	-	-
14	E2	+	-	-	-
15	E3	+	-	-	+

to be related to various diseases of man such as gastroenteritis. These findings strongly agrees with the publications of FAO/WHO (2003) which stated that in developing countries such as Sudan, Cholera, *Salmonellosis*, *Brucellosis*, *Shigellosis* and *Colibacillosis* are prevalent due to the feeding habit of people. The presence of *Staphylococcus aureus* and some enteric bacteria in the present study is in consonance with the findings of Gilbert & Harrison (2001) who reported that meat preserved with salt permit the growth of Staphylococcus aureus whereas, the presence of some members of *Enterobacteriaceae* is due to contamination from the intestines of slaughtered animals. Additionally, *Staphylococcus aureus* requires about 6.5% Sodium Chloride for growth and is usually found in salty meat products (Boles et al., 200). Many healthy people carry *Staphylococcus aureus* as a normal flora of the skin (Gilbert and Harrison, 2001). However, foods commonly implicated in *Staphylococcal* food poisoning are those that have been contaminated via physical handling and then subjected to time/temperature abuse (Food Safety Authority of Ireland, 2016).

The presence of *Escherichia coli* may be due to the use of non-portable water during washing of raw meat as stated by Umoh (2004). Bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp*, and *Shigella spp* are of public health importance they have been found to be related to various diseases of man such as gastroenteritis. In the present study, *Staphylococcus* aureus was recorded as the most frequently isolated bacteria. This is in conformity with the results of Egbebi et al., (2011), Tijjani et al., (2014); Lawrence et al., (2016); Nwakanma et al., (2015); Egbebi and Muhammed, (2016). Ananias et al., (2017) and Falegan et al., (2017) who also recorded *Staphylococcus aureus* as the most frequently isolated bacteria and highest percentage occurrence. However, this result is in disagreement with the findings of Moshood et al., (2012); Manyi et al., (2014) and Samuel et al., (2015) and Uze et al., (2012) who identified *Bacillus cereus*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella species*, and *Bacillus subtilis* and *Enterobacter aerogenes* respectively, as the most frequently *isolated bacteria*.

Additionally, it was observed in this study that *Escherichia coli* was the least isolated bacteria, and this is in agreement with the findings of Nwakanma et al., (2015); Lawrence et al., (2016) and Falegan et al., (2017) respectively. However, these findings were in disagreement with the findings of Edema et al., (2008); Ogbonna et al., (2012); Samuel et al., (2015) as well as that of Ananias et al., (2017) respectively, who reported *Salmonella spp*, *Shigella spp*, *Streptococcus pyogenes*, *Pseudomonas spp* and *Staphylococcus aureus* and *Lactobacter species* respectively as the least bacteria isolates recorded. The findings from this study shows that the level of hygienic practices of the beef tongue meat processing by the sellers affect the level of contamination of the meat, even though the results are within the range of satisfactory and marginal when compared with the guidelines of microbiological examination of ready-to-eat food samples. The critical control points of contamination of tongue meat are purchase of raw materials, storage of raw materials, slicing, spicing, roasting and serving (Edema et al., 2008). Therefore, control of contamination can be achieved if aseptic ways of preparation are observed.

4. Conclusion

The microbial quality assessment of beef tongue meat sold within Khartoum town showed that the Tongue meat was contaminated but within the satisfactory and marginal limits. The bacteria isolated include *Staphylococcus aureus*, *Escherichia coli*, *Shigella spp*, and *Salmonella spp*. The fungal isolates obtained from this study are *Penicillium spp*, and *Aspergillus niger*. The percentage of occurrence of bacterial isolates was highest in *Staphylococcus aureus* (43.5%), *Shigella spp* (21.7%), *Salmonella spp* (21.7%) and *Escherichia coli* (13.0%) recorded the lowest. It is concluded that the occurrence of such organisms indicates contamination of the Tongue meat samples. Hence, proper care in the course of preparation and handling of Tongue meat needs to be established.

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