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Microbial characterization of fermented meat products on some selected markets in the Kumasi metropolis, Ghana

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Fermented meat products are defined as meat that is inoculated with a microbial starter culture during processing under controlled condition, or meat allowed to ferment by natural fermentation meat microbial flora to give desirable characteristics. Fermentation of meat is mainly by lactic acid bacteria (LAB). Their biochemical characteristics project them as sugar fermentative, catalase and nitration reduction negative bacteria. This report is based on a preliminary research of isolation and characterization of microbes in fermented meat products on selected Ghanaian markets. The work specifically seeks to isolate and identify both essential and pathogenic microbes in fermented meat. Identification of microbial species is important for consumer protection and food law enforcement. In this study, samples of fermented bush meats and fermented sausages from selected Ghanaian markets were analyzed for their microbial content and load. Isolated microbes in samples were identified as Lactic acid Bacteria species, Streptococci species, Staphylococci species and Micrococcus species. Lactic acid Bacteria species (LAB) and micrococci species are characterized as essentially non pathogenic bacteria species whiles streptococci species and staphylococci species are characterized as the pathogen species. Isolates obtained from fermented bush meat were identified as LAB, staphylococci and streptococci species whiles isolates of fermented sausage were also identified as LAB and micrococci species. The total variable count for fermented sausage on an average was 1.3× 105 cfu, for smoked fermented bush meat the average loads was 2x 105 cfu whiles for fresh fermented bush meat the load was 2.5x 106 cfu.

Key words: Meat, bush meat, fermented meat products, food microbiology, food safety, Kumasi, Ghana.

INTRODUCTION

Meat is the edible part of domestic mammals, however, recent definition of meat has a broader meaning which encompasses all mammalian species, as well as fish, shellfish, poultry, and more exotic species such as frogs and alligators (Nakai and Modler, 2000). Similarly, meat also refers to animal tissue used as food, mostly skeletal muscles and associated fat but it may also refer to organs, including lungs, livers, skin, brains, bone marrow, kidneys, and a variety of other internal organs as well as blood (Hammer, 1987). Recent increase in the consumption of meat and its products arises from reasons includ-

ing high protein content, vitamins, minerals, lipids and savory sensation. Another group of meat is bush meat. Bush meat is the term commonly used for meat of terrestrial wild animals killed from subsistence or commercial purposes throughout the humid tropics of the Americas, Asia and Africa (http:// www.theonion.com/contents/ news). Species associated with bush meat are apes, other primates, ungulates, rodents and birds. Since, these animals are killed in the bush; natural fermentation preserves the meat and produces the characteristic flavour (Nout, 1994). A typical meat contains about 20% protein, 70% water, 5% lipid and 5% other substances e.g. carbohydrates, salts, vitamins etc. (Nakai and Modler, 2000). Meat is an excellent source of high biological value protein, vitamin B12, niacin, vitamin B6,

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Table 1. Standard microbial load specification in animal food product.

Grades	TVC (total viable count)/g at 30 ℃	Description
1	<1/2 million	Satisfactory
II	½ million to <10 million	Passable
Ш	10 million and more	Unsatisfactory

(Wilson et al., 1981)

iron, zinc and phosphorus. Meat is also a source of longchain omega-3 polyunsaturated fats, riboflavin, pantothenic acid, selenium and vitamin D. It is also low in fat and sodium.

Fermented meat products are defined as meat that is inoculated with microorganisms during processing under controlled condition to give desirable characteristics (Acton, 1977). However, most local manufacturers rely on natural fermentation without inoculation or any controlled condition. In such instances, the microbes present in these products come from the meat itself or the environment.

Meat is a highly perishable food. The rich source of nutrients of fermented meat provides both pathogenic and nonpathogenic microbes a suitable environment to grow during preparatory stage and storage (Steinkraus, 1994). Meat fermentation involves microbial processes in which carbohydrate is oxidized in the absence of oxygen with the release of ATP. The fermentative microorganism uses intermediate metabolite (pyruvate) as terminal electron acceptor instead of oxygen. These fermentative microorganisms are gram-positive, anaerobic, and acidophilic and are collectively termed lactic acid bacteria (LAB). Lactic acid bacteria are the most predominant microorganism found in fermented meat products. The genera mostly involved in meat fermentation include Lactobacillus, Pediococcus, Leuconostoc, Streptococcus and Enterococcus (Doyle et al., 2001). During fermentation in meat production, these groups of microorganisms are responsible for two biochemical steps occurring independently: Decreasing pH as a result of lactic acid production during glycolysis and formation of nitric oxide from nitrate to give the color of the product. Staphylococcus spp., Clostridium botulinum, Salmonella spp., Vibrio spp., and Escherichia coli are also sometimes found in some fermented meat products (Odunfa, 1998). Lactobaccili utilise the carbohydrate portion of the meat to produce acids and thus lower the pH, improving the texture of the products, providing stability against the proliferation of food pathogens and producing some aromatic compounds (Bacus, 1986). After fermentation of the carbohydrate portion, microbes then attack the proteins and fats. Beside lactic acid, there are a variety of other products that are found during the fermentation process. These include organic acids, carbon dioxide and alcohols that give the fermented products a distinct flavour and texture (Alcamo, 1997). The meat's pH is controlled by the quantity of dextrose added, fermentation temperatures, relative humidity and fermentation time (www.extension.osu.edu).

During the production of fermented meat products, commercial starter cultures are sometimes utilized. The United States Drug Administration, for example, stipulates that such a starter culture may contain microorganisms such as *Pediocococcus*, *Lactobacillus*, Plantarum and Micrococcus but not coagulase negative "staph".

Pathogenic bacteria and other microorganisms are known to be the most important food safety hazard associated with fermented meat product. Concerns have already been raised in the past about the role of meat and meat products in food poisoning. Records indicate that more than 74% of incidents of food poisoning are due to meat dishes (Hobbs and Robert, 1993). The International Committee on Microbiological Specifications for Food (ICMSF) has approved standards for the presence of pathogens on meat and their stipulated grades (Wilson et al., 1981). The ICMSF standard microbial load specification in animal food product is shown in Table 1.

Microorganisms involved in fermentation of meat are mainly gram-positive, anaerobic acidophilic and catalase negative (Egan, 1983). According to Brown, (2005), an unknown microorganism is identified by determining which genus it fits into. The Bergey's Manual of Determinative Microbiology uses the presumptive identification aids in the identification of microorganisms. In identification not all the biochemical tests must be employed.

Lactic acid bacteria are the most predominant microorganisms found in fermented meat products, but the rich source of nutrients of fermented meat provides suitable environment for the growth of food spoilage microorganism and pathogens. Pathogens that are likely to be found associated with fermented meat are Staphylococcus spp., C. botulinum, Salmonella spp., Vibrio spp., and E. coli.

Production of a safe and tasty fermented meat product is important to consumers and dependent ob both science and experience. In relation to science, the microorganisms involved in the fermentation of meat and their microbial load must be known. Each microorganism can be either beneficial or harmful to the consumer and even those that are beneficial, in large numbers can affect the consumer negatively. The objective of this study was therefore to culture and isolate micro-organisms of fermented meat products and to characterize the isolated microorganisms to determine the microbial numbers (load) of pathogenic and nonpathogenic microorganisms.

MATERIALS AND METHODS

In this study, fermented bush meats were obtained from three different markets; namely Asafo, Central and Adehyeman that sold fermented meat products in the Kumasi metropolis were randomly sampled. Five meat cuts from the same meat piece were sampled (fore and limb areas). These samples were from five different sellers of meat products. These samples were aseptically collected in sterile polythene pouches, sealed and transported in ice packs to the laboratory for microbial analysis immediately. This sampling procedure was repeated. Samples from each market was analyzed as one lot. Fermented sausage was also obtained from the Animal Science Department Kwame Nkrumah University of Science and Technology (KNUST), made from six month old pigs. The controls were fresh meat, sampled in the same manner as for the fermented meat from butchers selling meat in the same markets.

For the purpose of avoiding contamination from the working environment, all equipment, media test-tubes, Petri dishes were sterilized using one or a combination of the methods listed below: Equipments and media were sterilized in an autoclave at 121 °C for 15 min. Distilled water in test-tubes were also plugged with nonabsorbent cotton wool and sterilized by autoclaving

The inoculating chamber was sterilized by the use of UV radiation. Inoculation of culture was made an hour after the UV was switched off.

UV radiation does not penetrate surfaces; hence the working bench in the inoculation chamber was sterilized with concentrated ethanol. Detergent was used to wash equipment.

Inoculating loops and forceps were sterilized by heating to red-hot using Benson burner before and after use. Flaming was used to decontaminate the mouth of flasks, test-tubes and glass slides.

A hot-air oven designed to maintain an effective sterilizing temperature of 160 °C was used to sterilize Petri dishes enclosed in canisters and pipettes enclosed in a brass container.

Heat-labile materials were sterilized by filtration through bacteriological filter to prevent foreign microbial contamination. The liquid sugar for the fermentation test was sterilized by this method. Nutrient agar (NA), was generally used for the isolation of bacteria. This was prepared by 1000 ml of sterilized distilled water, 3 g of beef extract, 5 g of peptone, 15 g agar and 5 g NaCl. pH was then adjusted to 7.4.

In the isolation procedure, 1 g of each test sample was dissolved in 9 ml of peptone water and left to stand for 10 min. A loopful of each sample was picked and streaked on the sterilized nutrient agar media in Petri dishes. The plates were incubated at 37 °C for 24 h for colony formation.

Each colony formed on the plates originated from a single microbial cell and thus isolated in a pure form for further studies of interest. A turbid suspension of each colony with distinct morphological properties was prepared. Serial dilutions were made to 10-6 for each case using distilled water in sterilized test-tubes. For each case, 1 ml of the 10-6 dilution was pipetted, mixed with nutrient broth, heated and cooled to 45°C in test-tubes and poured into sterilized Petri dishes. The plates were incubated at 37°C for 24 h for pure culture formation. Pure colonies formed were subjected to identification to find which particular microorganism was present. For microbial load determination, 5.75 g of nutrient agar was weighed and dissolved in 250 ml of distilled water. The mixture was heated and stirred until a uniform mixture was obtained. The liquid agar was then autoclaved at a temperature of 121 °C for 15 min. The liquid agar was cooled to a temperature of 45℃. Each meat sample was diluted six times to reduce the microbial population sufficiently to obtain separate colonies during the plating. 1 ml of

each diluted sample was mixed with the liquid agar. The mixtures were poured into sterile culture Petri dishes and allowed to harden. They were then incubated at a temperature of 35 °C for 24 h. The isolated colonies that appeared after incubation were counted with the aid of a colony counter (Prescott et al., 2002).

Microorganisms growing on solid nutrient form colonies with distinctive morphology. Properties considered were colony form, elevation of colony and colony margin. Simple staining with crystal violet was carried on a smear of each isolated colony and viewed under the light microscope. The cells shape and arrangements of each colony were noted.

Gram's staining was also carried out on a sample of each colony that was picked, smeared and fixed on a slide in order to divide the microorganisms into gram-negative and gram-positive microorganism (Atlas et al., 1995).

Three biochemical tests namely, sugar fermentative, catalase and nitrite reductase test were conducted on each isolate to unearth their biochemical characteristics for identification.

Sugar fermentative test was employed to ascertain the presence of microbes that convert sugar to lactic acid. Three sugars: glucose, fructose and sucrose were used to prepare three separate media. The lactose broth media was prepared from 250 g cow meat, 1 litre of distilled water, 5 g NaCl, 10 g peptone, and 1 g yeast extract. The media was refrigerated for 24 h and after which it was steamed for 15 min and pH adjusted to 6.8-7.0. One part of lacrosse broth was dissolved in three parts of distilled water. Bromothymol Blue (BBT) indicator was added to give a greenish color. 1 g of each sugar was dissolved in 100 ml of lactose broth. 5 ml of the solution was pipetted in test-tubes and a Durham fermentation tube was inverted into each plugged test-tube. The test-tubes and their contents were sterilized at 121 ℃ in the autoclave. The tubes were inspected to ensure no gas was present in the Durham tube. In the transfer room, each medium was inoculated with a pure culture of each of the isolates. The cultures were incubated at 37 °C for 48 h to observe a yellow color change of the pH indicator and gas production.

Catalase test was used to detect the presence of the enzyme catalase which converts hydrogen peroxide to water and oxygen. Aerobic microorganisms are those that have this quality. A drop of 1% hydrogen peroxide was put on an agar slant to observe effervescence. The presence of the enzyme resulted in vigorous bubbling due to oxygen release.

Nitrate reductase test was used to detect the presence of the enzyme nitrate reductase in a microbial pure culture. These microorganisms use nitrate as terminal electron acceptor in their respiratory metabolic pathway by reducing nitrate to nitrite; a type of anaerobic respiration. The medium consisted of 0.2 g KNO3, 5 g peptone dissolved in 1000 ml of distilled water. Five ml of the solution was dispensed in test-tubes and autoclaved at 121 °C. One ml of pure culture was inoculated in each test-tube and incubated at 37 °C for 48 h. To each test-tube two drops of indicator A: Sulfanilic acid was added, followed by two drops of indicator β -dimethyl- α -naphtylamine (Atlas et al., 1995). A red color change indicated a positive result, and no color change indicated a negative result.

RESULTS

From Table 2, 12 isolates were obtained with each isolate having its own characteristics. Samples A, B, and C which were smoked fermented bush meat gave two and one distinct isolate respectively with isolate (1) of each sample showing a white color, bacilli in shape and a chained arrangement. Isolate (2) of sample A showed a yellow, cocci and chained arrangement. Samples A1, B1 and C1 which were fresh fermented bush meat gave two

Table 2. Colony characteristics.

Sample	Isolate	colony color	cell shape	Cell arrangement
Α	1	white	bacilli	chained
	2	yellow	cocci	chained
A 1	1	white	bacilli	paired
	2	white	cocci	chained
В	1	white	bacilli	chained
B1	1	white	cocci	clustered
	2	white	cocci	chained
С	1	white	bacilli	chained
C1	1	yellow	cocci	chained
D	1	white	bacilli	chained
	2	white	cocci	scattered
D1	1	white	bacilli	chained

A, B and C: smoked fermented bush meat; A1, B1 and C1: fresh fermented bush meat; D and D1: fermented sausages.

each for the first two and one isolate for the last sample. Isolate (1) of sample A1 gave a white, bacilli and paired arrangement, that of B1 gave a white, cocci and clustered arrangement and C1 gave a yellow, cocci and chained arrangement. Isolate (2) of both A1 and B1 gave a white, cocci and chained arrangement. Sample D and D1 which were fermented sausage gave two and one isolate respectively. Isolate (1) of both D and D1 gave white, cocci and chained arrangement with isolate (2) of D showing a white, cocci and scattered arrangement.

All isolates give a positive result with the Gram's staining procedure.

From Table 4, all isolates showed characteristic fermentative abilities by the positive sign, evidenced by the yellow color change of the Bromothymol Blue indicator and the void in the inverted vial of the Durham tube except isolate B1 (1) and C(1).

All isolates gave a negative result except isolate (2) of D which gave a positive result indicating the reduction of nitrate into nitrite by the isolate (Table 5). Again only isolate (1) of B1 and isolate (2) of D gave a positive result when the catalase test was performed (Table 6). A positive result indicates the presence of the enzyme catalase in these isolates while a negative result indicates the absence of the enzyme catalase.

Samples A, B, C, D and D1 from smoked fermented bush meat and sausage had microbial loads of 220,000 cfu (colony forming units)/g, 200,000 cfu/g, 180,000 cfu/g, 120,000 cfu/g and 140,000 cfu/g respectively and samples A1, B1 and C1 from fresh fermented bush meat a microbial load of 2,800,000 cfu/g, 2,600,000 cfu/g and 2,200,000 cfu/g respectively.

DISCUSSION

Fermented meat product is produced by the inoculation

of starter culture or allowing natural fermentation to give desirable characteristics. The morphological characteristics of individual microorganisms serve as preliminary criteria for identification. Of the several morphological properties, colonial characteristics and gram staining reactions were employed in the identification of isolates. In this research on microbial identification and characterization, twelve colonies of distinct morphological features, representative of the several thousands of colonies obtained with nutrient agar, were isolated and selected for further characterization. The selected isolates were bacilli and cocci in shape and were chained, clustered, scattered and paired in arrangement. Each isolate was subjected to various tests to study their characteristic features in order to identify them.

The Gram's stain is useful in microbial identification in that it helps to eliminate thousands of possible organisms. The positive result for all the isolates indicates that they retained the crystal violet dye.

The importance of biochemical tests in microbial identification is due to the production of certain metabolites unique to particular microbial family or species. Bacteria species that ferment sugar produce lactic acid and CO2. The acid lowers the pH of the lactose broth resulting in the change in the color of the basic indicator (Bromothymol Blue) in the sugar fermentative test.

With regards to the sugar fermentative test, each isolate showed characteristic fermentative abilities with the various sugars, evidenced by the color change of the media and the production of gas as revealed by the void in the inverted vial of the Durham tube. This confirms the production of lactic acid, characteristic of fermentative metabolism (Table 3). All the isolates gave a positive result with regard to one or more of the sugars, an indication that they are either anaerobic or facultative anaerobes.

The second biochemical test, the nitrate reduction test

Table 3. Sugar fermentative test.

Sample	Isolate	Glucose	Fructose	Sucrose
Α	1	+	+	+
	2	+	+	+
A1	1	+	+	+
	2	+	+	+
В	1	+	+	+
B1	1	-	+	+
	2	+	+	+
С	1	+	-	+
C1	1	+	+	+
D	1	+	+	+
	2	+	+	+
D1	11	+	+	+

A, B and C: smoked fermented bush meat; A1, B1 and C1: fresh fermented bush meat; D and D1: fermented sausages.

Table 4. Nitrate reductase test.

Sample	Isolate	Nitrate reduction
Α	1	-
	2	-
A 1	1	-
	2	-
В	1	-
B1	1	-
	2	-
С	1	-
C1	1	-
D	1	-
	2	+
D1	1	-

A, B and C: smoked fermented bush meat; A1, B1 and C1: fresh fermented bush meat; D and D1: fermented sausages.

is used to identify a group of microorganisms that posses the enzyme nitrate reductase. In the presence of the enzyme, nitrate reductase, the test reagent, KNO3 is converted to NO2 which reacted with the indicators suphanilic acid and naphthylamine to form nitrous derivatives with a characteristic red color. A positive result for this test indicates the use of nitrate as a terminal electron acceptor catalyzed by nitrate reductase. Of all the isolates only D (2), from fermented sausages, gave a positive result for this test (Table 4).

The last biochemical test, the catalase test is used to identify aerobic bacteria that posses the enzyme catalase that converts hydrogen peroxide to water and oxygen. This was characterized by bubbling on the slide containing the isolate. Only two isolates B1 (1), from fresh fermented bush meat, and D (2) showed a positive result

Table 5. Catalase test

Sample	Isolate	Catalase test
Α	1	-
	2	-
A1	1	-
	2	-
В	1	-
B1	1	+
	2	-
С	1	-
C1	1	-
D	1	-
	2	+
D1	11	-

A, B and C: smoked fermented bush meat; A1, B1 and C1: fresh fermented bush meat; D and D1: fermented sausages.

for this test (Table 5). This implies that the two isolates are aerobes and thus use the enzyme to decompose H2O2 produced in oxidative metabolism. The remaining isolates gave a negative results suggests that they are strict anaerobes and do not use oxygen as a terminal electron acceptor but rather nitrogen.

Microbial load indicates extend of microbial contamination of a sample. Both pathogenic and non-pathogenic have their standard load specification. Non-pathogenic microorganism when in amounts above recommended standards turn to pose some health hazards.

Samples A, B, C (smoked fermented bush meat), D and D1 (fermented sausages) had their microbial load being less than half a million colony forming units per gram of sample (cfu/g), while A1, B1 and C1 (fresh fermented bush meat) had their microbial load being more

Table 6. Microbial load content of meat samples.

Sample	TVC (total viable count) cfu/g at 37 ℃	log of cfu/g
Α	220,000	5.342
A1	2,800,000	6.447
В	200,000	5.301
B1	2,600,000	6.415
С	180,000	5.256
C1	2,200,000	6.342
D	120000	5.079
D1	140,000	5.146

A, B and C: smoked fermented bush meat; A1, B1 and C1: fresh fermented bush meat; D and D1: fermented sausages.

than half a million but less than ten million. With reference to the standard microbial load specification (Wilson et al., 1981), samples A, B and C fall in the satisfactory region. These samples are smoked fermented meat products, thus the smoking which was done at high temperature contributed to the reduction in microbial load since most bacteria are not able to withstand the high temperatures encountered in the smoking process. Fresh meats are sometimes contaminated with bacteria (Hobbs and Roberts, 1993; Burgess et al., 2005), which can be harmful to the human body (Lechowich, 1971). The major bacterial pathogens include: Salmonella, S. aureus, C. botulinum, Clostridium perfringens, B. cereus and E. coli (Hobbs and Roberts 1993). The sources of these microbes in meat could be inherent micro-flora in normal tissues of animals, air, environment, or contamination due to unhygienic slaughtering, handling and processing conditions (Zattola, 1972).

Soyiri et al. (2008) conducted a microbial assessment of fresh beef sold in a suburb of Accra, Ghana and found the beef samples were contaminated with Aerobic mesophiles (189-23000 cfu/g), S. aureus (22-59 cfu/g), B. cereus (17-41 cfu/g), C. perfringens (21-48 cfu/g) and E. coli (31-2200 cfu/g). The result of unhygienic practices and poor handling of beef by butchers was the major cause of contaminated beef.

Samples A1, B1 and C1 (fresh fermented bush meat), had microorganism loads in the passable region and this may due in part to the samples being fresh. Samples D and D1 which were fermented sausages gave microbial loads within the satisfactory region and this could be attributed to the hygienic conditions under which samples were prepared and also the smoking process that was employed. The Bergey's manual of determinative microbiology presumptive criteria was employed in the identification of all the isolates based on their morphological properties and unique biochemical characteristics.

Isolates A(1,2), A1(1), B(1), B1(2), C(1), C1(1), (from fresh fermented bush meat), D(1), and D1(1) (from fermented sausages)were determined to be Gram-positive, non-sporing, sugar fermentative, catalase negative, and

nitrate reduction negative, indicating Lactic Acid Bacteria (LAB) species.

Isolate A1(2) which was cocci and chained, Grampositive, catalase negative and nitrate reduction negative indicates a Streptococcus spp. This is a potentially pathogenic bacteria the presence of this bacteria has health implications. The Streptococcus spp. found may be due to contamination from the environment during the meat preparation. The group of pathogenic Streptococcus is differentiated from the group of non pathogenic Streptococcus by performing the test of hemolysis to differentiate the α-hemolytic, β-hemolytic, and non hemolytic Streptococci. Since the non hemolytic Streptococci rarely cause illness, the hemolytic strains are known to be the pathogenic strains (Hellerqvist et al., 1987; Vanderkooi et al., 2003; Yildiz et al., 2004). Antigenic tests are also used to classify the staphylococci strains into their Lancefield groups keeping in mind that most pathogenic strains belong generally to the Lanfield group A, B, C and G (Vartican et al., 1985; Hellerqvist et al., 1987).

Isolate B(1) which was cocci and clustered, Grampositive, catalase positive and nitrate reduction negative, indicates a Staphylococcus spp. Again this might be a pathogenic bacteria and its presence in the samples might be due to contamination from the working environment of the meat preparation and the sale point. Staphylococcus spp. is also present in the membranes of human noses, throats, hair Among and skin. Staphylococcus genus, the species S. aureus is known to be pathogenic but the remaining Staphylococcus strains are not necessary pathogenic. In order to discriminate between S. aureus and the "non S. aureus" the key tests of thermonuclease and staphylo-coagulase (and eventually if the need arises, the information of the tests by PCR amplification of the coagulase and nuclease gene). This however was not done in this work.

Isolate D1(2) which was cocci and scattered, Grampositive, catalase positive and nitrate reduction positive indicates *Micrococcus* spp. which is a LAB species.

From the identification, the microorganisms can be cha-

racterized as pathogenic and non-pathogenic.

It was noticed that A, A1, B, B1, C, and C1, which are fermented bush meat had their isolates identified as LAB, *Staphylococcus* spp. and *Streptococcus* spp. This can be attributed to the fact that in natural fermentation process, the production of lactic acid is low, a level which is not adequate to inhibit the proliferation of food pathogens.

The detection of potential pathogenic S. aureus means the samples do not meet the test of a Zero cfu/g which the Ghana Standards Board sets for fresh beef and beef products. Normally, pathogens in general should have a zero or no count in all ready to eat foods. Reference to the GSB and ICMS criteria may suggest that the pathogen levels in the beef are acceptable since they would have been destroyed after processing at high temperatures. This notwithstanding there is a risk of infection if virulent forms of this bacterium are present and the beef which is not well processed before consumption (Soyiri et al., 2008).

Isolate identified from sample D, fermented sausage (pepperoni) which was produced using a starter culture under controlled conditions, were predominately LAB and a specific Micrococcus species. The *Micrococcus* species is responsible for the red color of fermented sausage. During fermentation, Micrococceae produces the enzyme nitrate reductase that catalyses the conversion of added nitrate to nitrite and eventually nitric oxide. The nitric oxide then reacts with myoglobin to give the desired red color of the product.

Conclusion

From the various meat samples, twelve colonies were isolated which were bacilli and cocci in shape and were chained clustered, scattered and paired in arrangement. The isolates were identified as Lactic Acid Bacteria, *Staphylococcus* spp. *Streptococcus* spp. and *Micrococcus* spp.

REFERENCES

- Acton J (1977). The chemistry of dry sausages. Proceeding of Salmonella and Salmonellosis 1: 425-426.
- Alcamo IE (1997). Fundamentals of microbiology, 5th ed. Addison-Welsey, London, U.K. pp. 234-244.
- Atlas MR, Parks CL, Brown EA (1995). Laboratory manual to accompany microorganisms in our world. 1st ed. Mosby-Year Book Inc., New Tork, U.S.A. pp. 61-68
- Bacus JN (1986). Fermented meat and poultry products. Adv. Meat Res. 2:123-164.
- Bergey's manual of determinative microbiology. 9th ed. Lippincott Williams 7 Wilkins, Maryland, U.S.A.

- Brown EA (2005). Benson's microbiological applications laboratory manual in general microbiology, 9th ed. McGraw-Hill Companies, Inc., New York, U.S.A, pp. 249-260
- Burgess F, Little CL, Allen G, Williamson K, Mitchelli RT (2005). Prevalence of *Campylobacter, Salmonella*, and *Escherichia coli* on the external packaging of raw meat. J. Food Prot. 68(3): 469-475.
- Doyle PM, Beuchat RL, Montville JT (2001). Food microbiology, 2nd ed. ASM Press, Washington, DC, U.S.A, pp. 681-695.
- Egan AF (1983). Lactic acid bacteria of meat and meat products. Antonie Leeuwenhoek. 49: 327-336.
- Hammer GF (1987). Meat processing: ripened products. Fleischwirtschaft. 67: 71-74.
- Hellerqvist CG, Sundell h, Gettins P (1987) .Molecular basis for group B β-hemolytic streptococcal disease. Proceedings of the National Academy of Sciences of the United States of America ISSN 0027-8424 CODEN PNASA6.
- Hobbs BC, Roberts D (1993). Food Poisoning and Food Hygiene, 6th ed., St. Edmundsbury Press, Burry, Bodmin, Cornwall, London, UK, pp. 216-220.
- http://extension.osu.edu/~meatsci/as555/555.02fermentedlect.ppt. April 2009.
- http://www.theonion.com/contents/news. April 2009.
- Lechowich RV (1971). Microbiology of Meat: The Science of Meat and Meat Products, Price JF, Schweigert, BS, Freeman WH (Eds). San Francisco, USA pp. 230-235.
- Nakai S, Modler WH (2000). Food proteins, 1st ed. Wiley-VCH, Inc., New York, U.S.A pp.128-133.
- Nout MJR (1994). Fermented foods and food safety. Food Res. Int. 27: 291-298.
- Odunfa SA (1998). Review: African fermented foods. From art to science. Mircen J. 4: 259-273.
- Prescott ML, Harley F, Klein D (2002). Microbiology, 5th ed. McGraw-Hill Companies, Inc., New York, U.S.A pp. 659-710.
- Soyiri IN, Agbogli HK, Dongdem JT (2008). A pilot microbial assessment of beef sold in the Ashaiman market, a suburb of Accra, Ghana. Afr. J. Food Agric. Nutr. Dev. 8(1): 91-103.
- Streinkraus KH (1994). Nutritional significance of fermented foods. Food Res. Int. 27: 259-267.
- Tutenel AV, Pierard D, Van Hoof J, Cornelis M, L De Zutter (2003). Isolation and molecular characterization of Escherichia coli O157 isolated from cattle, pigs and chickens at slaughter. Int. J. Food Microbiol. 84(1): 63-69.
- Vanderkooi OG, Kellner JD, Andrew W, Wade AW, Jadavji T, Midgley JP, Louie T, Gregory JT (2003). Invasive *Streptococcus pneumoniae* infection causing hemolytic uremic syndrome in children: Two recent cases. Can. J. Infect. Dis. 14(6): 339–343.
- Vartian C, Lerner PI, Shlaes DM, Gopalakrishna KV (1985). Infections due to Lancefield group G Streptococci. Medicine (Baltimore). 64(2): 75-88
- Wilson NRP, Dyertt EJ, Hughes BR, Jones CRV (1981). Meat and meat products, factors affecting quality control, 5th ed. Applied Science Publishers Ltd., England pp. 81-108.
- Yildiz B, Kural N, Yarar C (2004). Atypical hemolytic uremic syndrome associated with group A beta hemolytic *Streptococcus*. Pediatr Nephrol. 19: 943–944.
- Zattola EA (1972). Introduction to Meat Microbiology, American Meat Institute, Chicago, USA. pp. 245-253.