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Microbial Quality of Preserved Sardines Sold in Mombasa

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Abstract

The research was conducted in five different market centres in Mombasa to investigate microbiological quality of sardines preserved using different techniques. A total of thirty (30) sardines were randomly sampled and purchased for analysis of pathogenic and spoilage microorganisms. The microbial load (bacteria and moulds) were determined using pour plate technique. The results showed that out of the six preservation techniques (smoking, drying, salting, freezing, frying and canning) sardines preserved using five of the techniques (83.3%) were contaminated with *Vibrio* species while two techniques (33.3%) allowed the growth of *Salmonella* species. Other bacterial species found in preserved sardines included *Micrococcus*, *Staphylococcu* and *Listeria* species among many others. There were also five species of fungi isolated from preserved sardines including *Aspergillus* species which is of clinical importance. The presence of pathogenic bacteria and fungi of clinical importance are some of the possible health risks that may be associated with consumption of preserved sardines sold in Mombasa. This may possess real health problems unless measures are put in place to prevent microbial contamination of preserved sardines. The total microbial contamination of sardine samples were compared to International Commission on Microbiological Specifications for Foods (ICMSF, 1998) shown in appendix I. Based on total microbial considering bacteria and fungi, smoked sardines had 3.57×10^3, dried 5.096×10^3, salted 2.528×10^3, frozen 3.74×10^2, fried 3.72×10^2 and canned 78 cfu. This showed that dried sardines were the most contaminated while canned were least contaminated.

Key words: Microbial quality; Preserved; Sardines.

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1. Introduction

Sardinella pilchardus (sardines) are small types of fish shorter than 20cm found in coastal regions. The species are coastal pelagic fish which prefer clear saline water. The species breed at all times of the year which contributes to their large numbers compared to other fish at harvest. They are among the most important commercial fish species due to their relatively cheaper source of animal protein for the population. Studies have shown that sardines are rich in Omega – 3 fatty acids which reduce occurrence of cardiovascular disease and lower blood sugar [10: 2447-2757]. Large quantities of sardines are harvested during rainy season along the beaches and are preserved by smoking, drying, frying, freezing, canning and occasionally salting. The demand for sardines is substantially high as the prices for beef, poultry and other fish species continue to increase.

Although sardines play important role in meeting food security and nutritional requirements among people of lower income, data on health risks associated with consumption of preserved sardines without cooking is lacking. The quality of preserved sardines like any other fish is linked to processing procedures. The preservation techniques and materials used, handling and packaging methods such as use of old news prints, cement papers and polythene bags are sources of microbial contamination [14: 443-453]. Several studies have reported on the incidences of pathogenic bacteria such as *Vibrio* spp., *Salmonella* spp. and numerous spoilage bacteria including *Listeria* and *Pseudomonas* bacteria among others which may cause major health problems to the consumers [16: 315]

The techniques used to preserve fish are all aimed at extending the shelf life of the fish under certain conditions. Smoking process involves application of wood smoke to impart smoky flavor on partially dry fish. The preservative effect of smoke is largely due to the presence of a range of phenolic compounds, nitrites and formaldehyde [2: 481-484]. The quality of smoked fish depends on processing and post – processing skills as well as the expertise of the fish processors and retailers [1:67 - 78]. On the other hand sun drying enhances the storage stability with minimized packaging requirements and reduced transport weights. However drying fish on the ground, rocks, palm leaves and sandy beaches contribute to the microbial contamination of sun dried fish thus jeopardize the quality and safety of the commodity.

Salting of fish is one of the oldest methods of preservation but, salted fish have been found to exhibit shorter shelf life [4: 91-98]. The method uses a lot of salt which do not attract many consumers. Salting of fish is therefore done on experimental basis or in small scale at home where other methods are unavailable. Freezing is a common practice in the fish and other animal protein based industry, because it preserves the quality for an extended time and offers several advantages such as insignificant alterations in the product colour, flavor and texture [15: 22-29]. Freezing at 0^{o} C inactivates bacteria, yeasts and moulds in preserved fish, but once thawed; these microorganisms can again become active, multiply under right conditions to levels that lead to human illness and spoilage of the product.

Fried fish are the dominant preserved seafood sold in the streets and are consumed without further preparation. There is general perception that fried fish vended may be unsafe for direct consumption because of the environment under which they are sold in the open near bus and matatu terminuses which expose them to

microbial and other contaminants. Mensah and his colleagues (2002) reported the presence of *Salmonella* and *Staphylococcus* in most street vended fish and meat in Accra Ghana. Most of the fried fish all over the world apart from the five star hotels reach the consumer already cold hence susceptible of microbial contamination [13: 148-155]. Canned seafood is the least used by consumers in Africa and its safety is generally not of concern to the consumers. The application of heat after the filling and sealing the cans is for complete destruction of all microorganisms. Occasionally some microorganisms enter the cans after the heat processing or their spores survive the heating process hence spoilage or food poison may occur. Due to such manufacturing defect, spore forming bacteria such as *Clostridium* spp. and Bacillus spp. may contaminate canned fish.

2. Materials and methods

Microbiological quality of preserved sardines was carried out in Mombasa between November 2008 and April 2009. Mombasa is located on the south eastern part of Kenya and consists of the island which is connected to the north by Nyali bridge, to the south by Likoni ferry, to the west by Makupa causeway through which runs the Uganda railway and Indian Ocean to the east. Mombasa lies between latitudes 3^o 80' and 4^o 10' S and longitudes 39^o 60' and 39^o 80' E, with a total land mass of 525 km^2 and inshore waters covering 65 km^2 [3: 231-242]. The town is known for its tropical climate which is warm and humid hence may encourage the survival of many microorganisms.

Sardines preserved using six different methods (smoking, drying, salting, freezing, frying and canning) were randomly sampled and purchased from five local markets (Kongowea, Mwembe tayari, Shibu street, Majengo and Likoni) in Mombasa town. The samples were collected from freezers in fish shops, trays and baskets used by fish mongers at the markets. A total 30 sardine fish were purchased in regular consumer dosage whereby the buyer selects the fish using a stick or spoon while the seller picks using hand and packs on available material. The samples for this study were collected in well labeled sterile polythene bags and transported to the laboratory at Kenya Marine and Research Institute at Mkomani near the Mombasa show ground to be analyzed for microbiological quality and safety.

2.1 Microbiological Analysis

All procedures for microbial analysis were carried out in a laminar – flow chamber and manual operations carried out using sterile disposable gloves. The samples were analyzed for aerobic plate count and yeast and mould counts. The aerobic plate count was enumerated using pour plate technique in selective media plate count agars using FDA (Food and Drug Administration) methods of food analysis which recommends the report of all aerobic plate counts computed from replicate plates. For each method of fish preservation 25 grams of sardines was homogenized in 225 ml of phosphate buffered saline which resulted to a dilution of 10^{-1} then, serially diluted up to 10^{-5}. From each dilution 0.1 ml were spread plated onto triplicate plates of selective media for the suspected bacteria and fungi. The plates were incubated at 30^o C for 48 h. and 25^o C for 5 days to culture bacteria and fungi respectively.

2.2 Determination of various bacteria

2.2.1 Vibrio species

Isolation and identification of *Vibrio* bacteria from sardines preserved using various methods was done using FDA methods of food analysis. This involved homogenizing 25 g of sardines preserved using the various methods (smoked, dried, salted, frozen, fried and canned) in 225 ml of phosphate buffered saline which gave dilution of 10^{-1}. The homogenate was left to stand for one hour for cells to float then, supernatant was used to prepare serial dilutions up to 10^{-5}. From each of the dilutions prepared 0.1 ml was spread plated onto triplicate plates of TCBS to culture *Vibrio* bacteria. The plates were incubated at 37^{o} C for 24 h. Greenish colonies were formed which confirmed the presence of *Vibrio parahaemolyticus* which were counted and computed for cfu/g for each of the methods used to preserve sardines. The colonies were also subjected to oxidase test to satisfy the presence of *Vibrio* bacteria which are oxidase positive.

2.2.2 Salmonella species

To determine the presence of *Salmonella* bacteria in preserved sardines, the homogenate was incubated overnight at room temperature, and then serial dilutions were prepared up to 10^{-5}. For every method of sardine preparation, 0.1 ml from each dilution was cultured on Tetrathionate broth media and incubated at 37^{o} C for 24 h. After the incubation period, loopfuls of the culture were plated onto triplicate plates of Brilliant Green agar (BGA) and incubated at 37^{o} C for 24 h. *Salmonella* colonies were identified as being grey and convex. The colonies were counted using ARTEK colony counter (U S) then geometric means calculated to determine cfu/g of sardines preserved using different methods.

2.2.3 Pseudomonas species

To determine presence of *Pseudomonas* bacteria in preserved sardines 25 g pieces of sardines preserved using different methods were homogenized in 225 ml of 0.1% peptone water to which salt had been added. Serial dilutions were then prepared up to 10^{-5} then, 0.1 ml from each dilution spread plated onto triplicate plates of Blood agar and incubated at 37^{o} C for 24 h. *Pseudomonas* colonies were determined by being gray-white and wrinkled. The colonies were counted for all plates followed by calculations of geometric means to determine cfu/g for each method of preservation.

2.2.4 Staphylococcus species

Staphylococcus bacteria in preserved sardines was determined by aseptically weighing 25 g of the fish into a sterile stomacher then, 225 ml of phosphate buffered saline were added and homogenized for 2 min. The homogenate was serially diluted up to 10^{-5} using Maximum Recovery Diluent (MRD) and from each dilution 0.1 ml was spread plated onto Baid Parker triplicate plates and incubated at 37^{o} C for 48 h. Black colonies surrounded by opaque zone encircled by clearing zone formed which were typical of *Staphylococcus*. All the colonies were counted and averaged for each dilution before calculating means to determine cfu/g for every method of preservation. The colonies were also subjected to catalase test using hydrogen peroxide which was positive.

2.2.5 Listeria species

Samples of 25 g from each method of preservation were aseptically weighed and homogenized in 225 ml of Buffered *Listeria* Enrichment Broth (BLEB, Merck and Darmstadt, Germany). The homogenate was incubated at 30^{o} C for 4 h. (Pre-enrichment), acriflavin 10 mg/l, sodium nalidixate 40 mg/l and cycloheximide (Sigma, St. Louis, U.S.A) were added as selective agents [18: 51-60]. The pre-enrichment culture was serially diluted up to 10^{-5} then triplicate plates of PALCAM agar (Oxoid, United Kingdom) were spread plated with 0.1 ml from each dilution. The plates were incubated at 30^{o} C for 48 h. then sub cultured onto Tryptone Soy Yeast Extract agar and incubated at 35^{o} C for 24 h. Black colonies with halo were formed typical of *Listeria* which were counted from all the plates and averaged for each dilution and subsequently geometric means calculated to determine cfu/g for every method of preservation.

2.2.6 Escherichia coli species

Presence of *E. coli* in preserved sardines was determined by plating extracts from the fish specimen on Eosin Methylene Blue (EMB) agar. 25 g of sardines preserved using different methods were aseptically weighed into sterile stomacher then 225 ml of Phosphate buffered saline were added and blended for 2 min. Serial dilutions were then prepared from the homogenate up to 10^{-5} and then 0.1 ml inoculated into 3 LST (Lauryl Tryptose) tubes. The tubes were then incubated at 44^{o} C for 24 h. then checked for gas production. Loopfuls of the gassing suspensions were streaked onto EMB agar and incubated at 35^{o} C for 24 h. Nucleated colonies typical of *E. coli* were observed, counted on all plates and means worked out for cfu/g of sardine preserved using different methods.

2.2.7 Proteus species

Samples of 25 g preserved sardines were crushed and homogenized in 225 ml of phosphate buffered saline. Serial dilutions were prepared from the homogenate up to 10^{-5} and from each dilution 0.1 ml were inoculated onto triplicate plates of nutrient agar and incubated at 37^{o} C for 24 h. Gram stain and oxidase tests were carried out to check for presence of *Proteus*. The colonies that satisfied the criteria were sub cultured onto MacConkey agar in which 3% sodium chloride had been incorporated to prevent swarming of *proteus* cells then incubated at 37^{o} C for 48 h. according to method described by [5:4307- 4314]. *Proteus* colonies were determined as being pink and counted on all plates, averaged for each dilution them means calculated to find cfu/g.

2.2.8 Aeromonas species

25 grams preserved sardines were homogenized in 225 ml of alkaline peptone water then serially diluted up to 10^{-5} using buffered phosphate diluents (BPD). From each dilution 0.1 ml was spread plated onto starch ampicillin agar (SA) triplicate plates and incubated at 28^{o} C for 24 hours. Colonies of honey – brown colour formed which were typical of *Aeromonas* hence were counted on all plates and means worked out to determine cfu/g.

2.2.9 Micrococcus species

Micrococcus bacteria in preserved sardines were determined by homogenizing 25 g of the preserved fish in 225 ml 0.1% peptone water followed by serial dilutions up to 10^{-5}. The serial dilutions were allowed to stand for 30 min. for cells to float then measurements of 0.1 ml were inoculated onto Furoxone Trypticase Oil red (FTO) agar and incubated at 37^{o} for 24 h. Yellow colonies typical of *Micrococcus luteus* were formed which were counted on all plates, averaged for each dilution then geometric means calculated for cfu/g of sardine for all preservation methods studied.

Other bacteria isolated from preserved sardines included *Streptococcus* species which were determined by formation of pink colonies on Streptococcus agar and *Plesiomonas* species which formed yellow colonies on Brain Heart Infusion (BHI) agar.

2.3 Fungal species from preserved sardines

Preserved sardines were also found to harbor various strains of fungi which are important to the public health regulations. The fungi were identified using selective media for the suspected moulds and yeasts. 25 grams of preserved sardines were homogenized in 225 ml of 0.1% peptone water then serially diluted to 10^{-5} dil. From each dilution 0.1 ml of homogenate were inoculated onto triplicate plates of selective media for the suspected fungi.

2.3.1 Penicillium species

To determine presence of *Penicillium* species the homogenates were allowed to stand for 10 min. so that spores could float [9: 66-675]. The preculture was then serially diluted before 0.1 ml spread plated onto Czapeck media triplicate plates. The plates were incubated at 25^{o} C for 5 days. *Penicillium* colonies were identified as being dark green and counted on all plates. The average per dilution was worked out followed by geometric means to find cfu/g.

2.3.2 Rhizopus species

To determine *Rhizopus* species, serial dilutions of the homogenates were inoculated onto triplicate plates of YM – 11 agar and incubated at 25^{o} C for 5 days. Grey colonies typical of *Rhizopus* species were observes and counted on all the plates. The colonies were averaged for every dilution then means were calculated for cfu/g of sardine preserved using different methods.

2.3.3 Cladosporium species

The homogenates were serially diluted then inoculated onto triplicate plates of potato dextrose agar (PDA). The plates were incubated for 5 days at 25^{o} C then observed for olive green colonies. Such colonies were counted on all the plates, averaged per dilution and then means worked to determine cfu/g.

2.3.4 Aspergillus species

To determine the presence of *Aspergillus* species in preserved sardines, homogenates were prepared by blending 25 g of the fish in 225 ml of 0.1% peptone water followed by serial dilutions of the homogenates up to 10^{-5}. Triplicate plates of potato dextrose agar were spread plated with 0.1 ml from each dilution. The plates were incubated at 25^{o} C for 5 days. Yellow – brown colonies typical of *Aspergillus* on PDA formed which were counted on all the plates and geometric means calculated for cfu/g.

2.3.5 Fusarium species

Homogenate of preserved sardines were prepared by blending 25 g of the fish in 225 ml of 0.1% peptone water. The homogenates were then serially diluted up to 10^{-5} and from each dilution triplicate plates of Czapeck media were spread plated with 0.1 ml of homogenate then incubated at 25^{o} C for 5 days. Purple colonies were observed and counted on all plates followed by working out of geometric means to determine cfu/g.

2.4 Data Analysis

Data analysis from this study was carried out using computer package MINITAB version 13.0 to establish significant differences in the number of colonies recorded from sardines preserved using different methods.

3. Results

3.1 Microbiological quality and safety of preserved sardines sold in Mombasa-Kenya

In Mombasa –Kenya, Common seafood preservation techniques used by fishermen are: Smoking, Drying, Salting, Freezing, Frying and Canning. Microbial counts of bacterial and Fungal contaminations were established in Sardines preserved using these techniques and recorded.

3.2 Bacterial colonies found in preserved sardines

The findings established that the common types of bacteria isolated from preserved sardines in Mombasa were; *Vibro sp, Salmonella sp., Listeria sp*., *Staphylococcus sp*, *E. coli*, *Pseudomonas sp*., *Aeromonous sp.*, *Proteus sp*., *Streptococcus sp*., *Micrococcus* and *Plesiomonous sp*.

3.3 Types of bacterial species in preserved sardines

Based on the six preservation techniques tested, the types of bacteria sp. that were prevalent in most (5) preservation techniques out of [6: 169] was *Vibrio sp*. *Staphilococcus sp.* and *Micrococcus sp.* which had prevalence of 83.3% as shown (Table 1). Bacteria sp. *Pseudomonas sp., Streptococcus sp.* and *Proteus sp.* had the lowest prevalence (16.7%). *Pseudomonas sp.* was only found in frozen Sardines while *Streptococcus sp.* and *Proteus sp.* were found only in fried sardines.

Table 1: Prevalence of the bacterial species on Sardines preserved using various methods

Type of Bacteria	Total cfu	Rank (1- Most prevalent)
Vibrio sp.	83.3%	1
Salmonella sp.	33.3%	4
Listeria sp.	66.7%	2
Staphilococcus sp.	83.3%	1
Pseudomonas sp.	16.7%	5
E. coli	50%	3
Aeromonas sp.	33.3%	4
Proteus sp.	16.7%	5
Streptococcus sp.	16.7%	5
Micrococcus sp.	83.3%	1
Plesiomonas sp.	33.3%	4

Source: Research 2011

Number of bacterial colony forming units found in the preserved sardines was as shown in table 2.

Table 2: Total colony forming units (cfu) of bacteria isolated in Preserved Sardines in Mombasa

Type of Bacteria	Total cfu	Rank (1- Most prevalent)
Vibrio sp.	132	3
Salmonella sp.	20	9
Listeria sp.	118	6
Staphilococcus sp.	298	2
Pseudomonas sp.	27	8
E. coli	38	7
Aeromonas sp.	19	10
Proteus sp.	10	12
Streptococcus sp.	11	11
Micrococcus sp.	8304	1
Plesiomonas sp.	126	4
Total cfu	**9104**	

Source: Research 2011

Total bacterial colony forming units in the various types of sardines preservation techniques were therefore established as shown (Table 2). There was no significant difference in the total bacteria cfu found in the six preservation methods ($P > 0.05$).

Table 3: Total bacteria cfu in the Sardines preserved using various preservation techniques

	Smoked	Dried	Salted	Frozen	Fried	Canned
Total bacteria (cfu)	2547	4021	2170	158	135	73
Mean cfu ± SE	636.75 ± 568.2	804.2 ± 769.6	310.0 ± 283.1	31.6 ± 9.36	16.86 ± 6.46	23.5 ± 21.5

Source: Research 2011

3.4 Prevalence of fungal species in preserved Sardines sold in Mombasa

Fungal species identified from the preserved sardines were; *Penicillium sp.,Rhizopus sp., Aspergillus sp., Fussarium sp.* and *cladosporium sp.* Most prevalent fungi species was found to be *Penicillium spp.* which was found in five out of the six fish preservation techniques tested. The research showed that *Aspergillus sp.* was only found in dried sardines.

Table 4: Fungi prevalence in the six preservation techniques tested

Type of Fungi	Prevalence (%)	Rank (1- Most common among preservation techniques)
Penicillium sp.	83.3%	1
Rhizopus sp.	66.7%	2
Aspergillus sp.	16.7%	4
Fussarium sp.	33.3%	3
Cladosporium sp.	66.7%	2

Source: Research 2011

The total number of colony forming units (cfu) found in the preserved Sardines sold in Mombasa are as shown in table 5.

Table 5: Total fungi colony forming units (cfu) of fungi isolated in Preserved Sardines in Mombasa

Type of Fungi	Total cfu	Rank (1- Most common)
Penicillium sp.	481	2
Rhizopus sp.	1200	1
Aspergillus sp.	13	4
Fussarium sp.	20	3
Cladosporium sp.	1200	1
Total	**2914**	

Source: Research 2011

When considering the individual preservation techniques used by the fishermen, the total fungal colony forming units found in Sardines preserved using smoking techniques were higher than the fungi colony forming units found in all the other preserved techniques.

There was no significant difference in the total fungal colony forming units found in the six preservation methods ($P > 0.05$).

Table 6: Total Fungi cfu in the Sardins preserved using various preservation techniques

	Smoked	Dried	Salted	Frozen	Fried	Canned
Total Fungi (cfu)	1023	1075	358	216	237	5
Mean cfu ± SE	341.0 ± 170.8	215 ± 115.3	119.3 ± 42.7	216.0 ± 0.00	79.0 ± 2.10	5.0 ± 0.00

Source: Research 2011

Preserved Sardine fish quality based on the Bacterial and fungal load was therefore found to be higher in dried and smoked fish. These had higher numbers of bacteria and fungi cfu respectively. Figure 1.

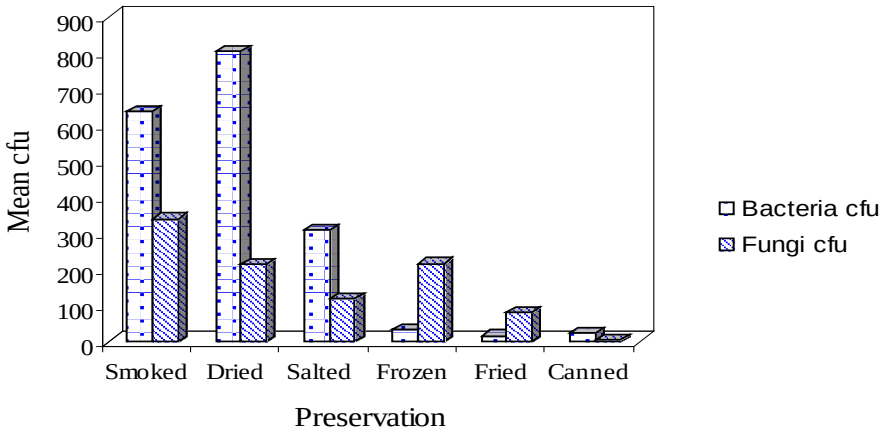

Figure 1: Bacterial and fungal colony forming units found in Sardins preserved using different techniques

4. Discussion

4.1 Prevalence of bacterial species in sardines preserved using various techniques

The results from the study showed that preserved sardines sold in Mombasa were contaminated with various species of bacteria and fungi. The total counts of bacteria from the six preservation methods studied ranged from

(73 cfu) and (4021 cfu). *Vibrio* sp. contaminated salted sardines more than those preserved using other methods and is attributed to the fact that *Vibrio* requires salt for its growth. The appearance of the organism in five out of the six methods studied could have been caused by the humid saline environment which would induce the growth in any substrate. *Salmonella* bacteria were isolated from dried and salted sardines although ($<10^2$) could have resulted from improper handling or sale under unhygienic conditions. At the same time *Staphylococcus* bacteria also occurred in five out of the six methods of preservation because the organisms are adapted to growth in salty environments where most bacteria cannot survive (Nketsia, 2007). *Micrococcus* sp. was the most prevalent in preserved sardines especially from dried sardines (3882 cfu) but was lowest in frozen (19 cfu). *Listeria* sp. was highest in sardines preserved by smoking which stays longer in stores. Many studies have documented abundance of *Listeria* in fish with extended shelf life [7: 122-132], although the organisms were not isolated from frozen and canned sardines. *Pseudomonas* bacteria were only found in frozen sardines showing lower counts as a result of good sanitation in the fish shops. Dalgaard and his colleagues 1993 found that when iced fish are stored aerobically, they get spoilt by when bacterial counts reach 10^7cfu/g and that at such levels the flora is composed almost exclusively of *Pseudomonas* sp. Various studies have established the organism as specific spoiler of iced fish [8: 303-316]. *E. coli* was isolated from dried, salted and fried sardines and this could be due to unclean handling procedures in stores or the markets. The organism was not isolated from smoked sardines which could be due to phenolytic compounds on the surface of the fish acting as antibiotics to the cells. At the same time E. coli could have failed to grow in frozen and canned sardines due to extreme temperatures in freezer and lack of oxygen in canned sardines. *Proteus* sp. and *Streptococcus* sp. showed the lowest total counts from sardines and could be due to poor handling and unhygienic market or package conditions because their growth is normally inhibited by oil due to the lack of oxygen [17: 13]. *Aeromonas* sp. appearance in frozen and fried sardines may be attributed to handling and market contamination. *Plesiomonas* sp. is a new opportunistic foodborne pathogen with little documentation [12: 449] but, was isolated from salted, frozen and canned. The contamination could have occurred after processing or after opening the tin for the case of canned sardines.

4.2 Total colony forming units of bacteria isolated from preserved sardines sold in Mombasa

The results showed that preserved sardines were contaminated by bacterial species at different levels. There were significant differences in the level of contamination as shown (Table 2). This comprised of eleven genera of bacteria isolated and identified. *Micrococcus* sp. was the most prevalent contaminant (8304 cfu) while the least were *Streptococcus* and *Proteus* (11 and 10) respectively. The total bacteria colony forming units in sardines preserved using various techniques were also worked out and recorded (Table 3). Dried sardines had the highest bacterial load (4021 cfu) followed by smoked and salted (2547 cfu) and (2170 cfu) respectively. There was no significant difference in bacterial contamination of frozen, fried and canned sardines although (73 cfu) for canned sardines was lower than in frozen (158 cfu) and (135 cfu) in fried.

5. Conclusion

To evaluate the microbial quality of the samples studied the results were compared to ICMSF (International Commission on Microbiological Specification for Foods, 1998) which is also accepted by FDA. The results constitute an indicator of microbiological contamination of preserved sardines sold in Mombasa. The overall

microbial quality of preserved sardines used in the study was within acceptable limit compared with ICMSF (1998) which put spoilage at level exceeding 10^6. The presence of *Vibrio* and *Salmonella* species in preserved sardines does not necessarily have effect on the safety of the products if the commodity is heated up to 65^o C before consumption to kill the bacterial cells. However the presence of fungi species especially *Aspergillus* requires that public health department should pay urgent attention to prevent them from contaminating fish to protect consumers from aflatoxin poisons. *Aspergillus* were isolated only from dried sardines hence, surveillance should be put in place for all dried fish to detect mycotoxins.

Microbial contamination of sardines preserved using different techniques revealed that dried sardines were the most contaminated having total bacterial count (4021 cfu) and fungal (1075 cfu) followed by smoked with total bacterial (2547 cfu) and fungal count (1023 cfu). This requires that fish preserved using these two methods must be properly cooked before consumption. Canned sardines recorded the lowest microbial count with bacterial (73 cfu) and fungal (5 cfu) which could have occurred after the tins were opened so, such sardines may be consumed without further cooking. This does not mean canned sardines are safe for direct consumption unless further analysis is done using samples from various towns in Kenya.

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APPENDIX I

Recommended Microbial Limits for Saefoods (ICMSF, 1986, 1998)

Table 7

	Minimum limit/g	Maximum limit/g
Dried fish	10^4	10^7
Smoked fish	10^4	10^7
Salted fish	10^2	10^7
Frozen fish	10^5	10^7
Fried fish	10^4	10^4
Canned fish	Nil	Nil