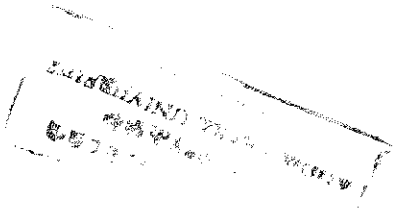
ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

MICROBIOLOGICAL PROFILE OF IMPORTED AND LOCAL BRANDS OF CANNED FOODS IN ADDIS ABABA

BY

YONAS CHEKOL BELAY



A THESIS PRESENTED TO THE SCHOOL OF GRADUATE STUDIES OF THE ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN BIOLOGY

JUNE, 2006

DEDICATION

This work is dedicated to my father, Chekol Belay.

ACKNOWLEDGEMENTS

First, I would like to thank and praise God for giving me endurance to complete the study and for his will that made this possible.

Next I would like to express my deepest gratitude to Prof, Mogessie Ashenafi, my advisor for his willful help constant guidance and encouragement I gratefully received through out the research period.

I would like to acknowledge school of Graduate Studies, Department of Biology, Addis Ababa University for offering me this opportunity of my study.

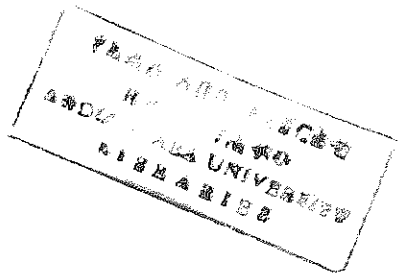
I also would like to express my thanks to Prof. Aklilu Lema institute of pathobiology for providing me laboratory facilities and other materials required for the work. I also appreciate the contribution of all staff of the institute that involved for the success of this thesis, especially Ato Haile, Ephrem and Mulugeta.

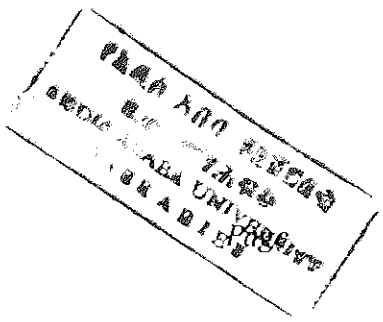
From the depth of my heart, I express my big thanks and appreciation to my fiancé Aynalem Metaferia for here care, moral support and encouraged me throughout the study period.

I can't find words to thank Ato Daniel Eressa for his Laptop, which made the write up much easier

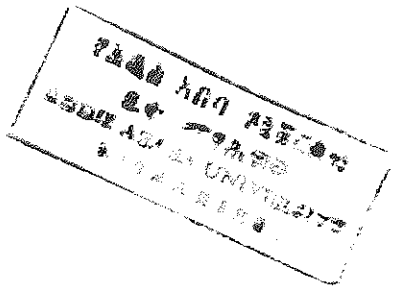
Lastly, I want to thank all my warmest thanks go to my families, friends, relatives and colleagues who encouraged me throughout the study period

TABLE OF CONTENTS





LIST OF TABLES

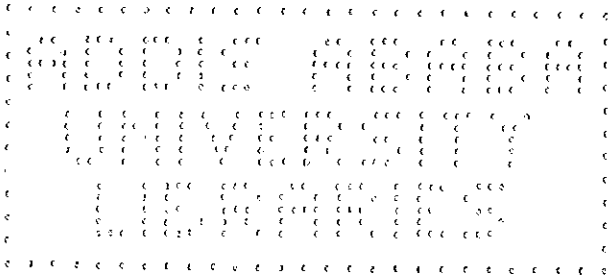


LIST OF FIGERS

Page

LIST OF ABBREVIATIONS

ALAR	As low as reasonably achievable
ALOP	Appropriate Level of Protection
Aml	Amoxycillin
Amp	Ampicillin
APHA	American Public Health Association
Cep	Cephalothin
CFPRA	Compden Food Preservation Research Association
Chl	Chloramphenicol
CMC	Sodium-Carboxy-methyl Cellulose
Ery	Erythromycin
FDA	Food and Drug Administration
Gen	Gentamycin
IFT	Institute of Food Technologists
Kan	Kanamycin
Met	Methicillin
NCCLS	National Committee for Clinical Laboratory Standards
NG	No Growth
Pen G	Penicillin G
Pol B	Polymyxin B
PRC	Peoples' Republic of China
SHZ	Slow heating zone
Str	Streptomycin
Sxt	Sulfamethoxazole-Trimethoprim
Tet	Tetracycline
USA	United States of America
USFDA	United States Food and Drug Administration
Van	Vancomycin



ABSTRACT

The primary objective of this study was to evaluate the microbiological quality and safety of canned foods available to consumers on the market in Addis Ababa and also to assess the fate of contaminating pathogens in the canned foods when stored at ambient and refrigerator temperature. The distribution of drug resistance among the dominant isolates was also evaluated. A total of 100 samples of canned foods comprising 20 each of, fish, meat, vegetables, milk and soups were collected from Addis Ababa super markets and shops. Date of production, expiration and ingredients of each sample were recorded. The samples were bacteriologically analyzed for counts of aerobic mesophilic, *Staphylococcus* spp, *Coliforms* and *yeast*. The pH of each sample was determined from the original sample. The aerobic mesophilic bacteria were dominated by, *Bacillus* spp, *Micrococcus* spp and other Gram-positive rods. *Staphylococcus* spp and *Coliforms* were encountered in some fish and meat canned food products. *Salmonella* and *Shigella* spp were not detected in any of the 100 samples. A total of 256 aerobic spore-forming bacilli were isolated from canned foods. *B. brevis, B. cereus, B. licheniformis, B. larvae, B. firmus, B. macerans B. pumilus and B subtilis* were the frequent isolates. Almost all *Bacillus* isolates were susceptible to chloramphenicol, erythromycin, gentamycin and kanamycin. Most isolates exhibited resistance to amoxycillin and ampicillin. Of the isolates tested for antibiotic sensitivity profile 139, (53%) showed multiple antibiotic resistance. Growth of *Shigella flexineri* and *Salmonella* Typhimurium inoculated into commercially available canned foods (meat, fish, vegetable and soup) was studied at refrigeration and ambient temperature. In both temperatures the strains grew and multiplied luxuriously

1. INTRODUCTION

Shelf stable canned foods are packed in hermetically scaled containers and are commercially sterile. Commercial sterility of thermally processed food means the condition achieved by the application of heat alone or in combination with other treatments to render the food free from microorganisms capable of growing in the food under normal conditions of distribution and storage.

Currently there are various types of canned foods produced worldwide. These include canned meat and vegetable salads, powdered milk, canned baby foods, mayonnaise and salad dressing products, pickles, jams, jellies and related products, canned soups, canned meat and poultry products, canned sea food products, canned dry pack products, canned juices, fruit drinks and water, canned fruits and canned vegetables.

Canning, as we know it today, was developed when a retort (pressure cooker) was invented in 1860. This equipment made use of steam under pressure allowing heating of canned food to 121°C. Since the food is sealed before the can is heat processed, the food is not exposed to contamination after processing and, in such case; the can is referred to as a sanitary can.

Despite this fact, canned foods are subject to microbial spoilage. The range of food products that are thermally processed is very diverse. It can include low, medium and high viscosity liquids, some with particulates (Ranken, 1997). The effect of these different substrates on the heat resistance of a microorganism can be quite marked, with some proteins, fats and high total solids increasing the heat resistance by a factor of 2 or 3 when compared with the standard heat resistance of a similar microorganism in a broth (Gaze, 1992). The influence of these components on the heat resistance of microorganisms must, therefore, be carefully considered during product development.

Spoilage of canned foods is usually caused by growth of microorganisms following leakage or under-processing. Leakage occurs from can defects arising from punctures or rough handling. Contaminated cooling water sometimes leaks to the interior through pinholes or poor seams and

introduces bacteria that cause spoilage. A viable mixed microflora of bacterial rods and cocci is indicative of leakage, which may usually be confirmed by can examination (Warren *et al.*, 1998)

Under-processing may be caused by undercooking, inaccurate or improperly functioning thermometer, excessive contaminations of the product for which normally adequate processes are insufficient, and product resulting in a more viscous or tighter packing in the container. Under-processed and leaking cans are of major concern and both pose potential health hazards (Warren *et al.*, 1998).

Based on acid content, canned foods are categorized as high acid, acid and low acid products. High acid canned foods include fruits and fruit and vegetable products, whose pH ranges from < 3.7 - 4.0, acid canned foods have pH 4.0 - 4.6 and low acid canned foods have pH > 4.6. The pH values of low-acid canned foods provide an optimum pH for reproducing, outgrowth of spores and toxin production by bacteria. Canned foods that are considered to be low acid are meat and poultry products, vegetables, fish and milk products. These canned foods require proper handling of raw materials during production, adequate thermal treatment and proper handling of the product until it reaches consumers. The need for time/temperature control is primarily important to determine the potential for the survival of pathogenic microorganisms of concern, and the potential for the subsequent growth and/or toxin production (IFT/FDA, 2003). Improper heat treatment and process practices make these foods susceptible to bacterial spoilage.

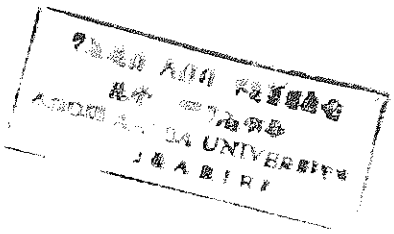
The main assets of early conventional models applied in the field of canned food industries were their simplicity and robustness due to application of high temperature, regardless of the nature of microbial populations concerned. One of the most important limitation is only that temperature is considered for the evaluation of microbial heat resistance while it is generally recognized that some other prominent factors such as pH and a_w of heating medium greatly affect the survival of heated cells (Mafart, 2000).

Canned foods have also been involved in enteric infections and food poisoning incidents, including cases of typhoid fever, botulism, salmonellosis and staphylococcal poisoning

(Gonzalez *et al.*, 2002). Problems had occurred related to spoilage of consignments of canned foods from a variety of countries (codex, 1983 cited in Gonzalez *et al.*, 2002).

The main objective of this study was, therefore, to evaluate the microbiological quality and safety of canned foods available to consumers in grocer's shops and supermarkets in Addis Ababa. The study would assess the fate of contaminating pathogens in the canned foods at different storage temperatures and evaluate the distribution of drug resistance among the dominant bacterial groups isolated from the canned foods.

2. LITERATURE REVIEW



2.1. Historical aspect of safe food production

The need to produce safe food has a long history. Food borne diseases have been among the major problems in activities such as hunting, food-gathering, and domestic production of animals and crops. Ancient Egyptians used drying as a precaution to preserve their grains. Attempts to preserve other foods were based mainly on experience gained in associating the spoilage of a food with the manner in which it had been prepared and stored. Increasingly, it became clear that a safe condition could only be maintained if the product was kept dry and away from contact with air. Some foods were treated with honey and later with olive oil (Hartman, 1997). Such activities led to the development of additional preservative measures such as heating and salting.

Most of the food safety requirements of early mankind were established thousands of years ago when religious laws were likely to have been the only ones in existence. Food control measures in civil law were introduced in much later date. Since the underlying causes of food borne illnesses were unknown, microbial food poisoning was recurrent. However, the situation changed after 1795, when the French government, driven by war, offered a substantial reward for any one developing a new method of preserving food. It was Nicholas Appert, a Parisian confectioner, who accepted the challenge and developed a wide-mouth glass bottle that was filled with food, corked and heated in boiling water for abou six hours (Hartman, 1997). In 1810, Durand in England patented the use of tin cans for thermal processing of foods, but neither Appert nor Durand understood why thermally processed foods did not spoil despite the fact that, in 1677, Van- Leeuwenhoek had discovered his little heat-sensitive animalcules (Dubell, 1960).

It was Louis Pasteur who provided the scientific basis for heat preservation in the period 1854-1864 (Hartman, 1997). During this time, he showed that certain bacteria were either associated with food spoilage or caused specific diseases. Based on Pasteur's findings, commercial heat treatment of wine was first introduced in 1867 to destroy any undesirable microorganism, and the process was described as "Pasteurization". Over the next 100 years or more, laboratory isolation

and study of pure cultures of microbes remained among the predominant activities of food microbiologists (Hartman, 1997).

2.2 Establishment of Process Criteria

At the start of the twentieth century, it was already recognized that protection of the public against food borne hazards required proper control of heat treatment used commercially in food production. The first mathematical evaluation of the heat sterilization process for canned foods was made by Bigelow *et al.*, (1920) and later developed by Ball (1923) to derive methods for calculating the time necessary to process canned foods at appropriate temperature. For commercial sterilization, the goal of thermal processing was to reduce the probability of survival and growth of microorganisms in a particular canned food to an acceptably low level. The starting point for the rational of what is now termed as 'an appropriate level of protection' (ALOP) was the work of Esty and Meyer (1922).

2.3 Canning process

Canning process involves various steps. Each pre- and post-canning step has significant role in attempting the quality of the canned foods. These canning operations are inspection of incoming raw materials, food preparation, blanching, filling the container, exhausting, sealing the container, heat processing, cooling, incubation and quality control checks.

2.3.1. Pre Canning process

Inspection of incoming raw materials: Raw materials are separated and sorted depending on their color, texture, off odor.

Food preparation: This step involves thawing, cleaning, washing, sorting, grading, peeling, trimming, slicing or dicing for vegetables and fruits. Meats and fish may be tempered, boned, trimmed, diced, minced or sliced, etc. before the food is subjected to the next process.

Blanching: Blanching is a mild heat process applied to foods for a time period. The aims of blanching include the following: Shrink the product and release respiratory gases, inactivate

enzymes, aid other processes such as peeling, cutting, dicing, etc., lower microbial load, set and fix colour, remove raw flavors or surface materials which may lead to off flavors. Blanch efficiency is determined by checking the activity of the peroxidase enzymes.

***Filling the container*:** At this stage the food is placed into the container. Filling should not be under or over the required volume. Under-filling gives a large headspace. Large headspaces result in mushiness of the contents due to excessive movement inside the can. Over-filling produces excessively small headspaces. Over-filling causes under processing if the process depends on agitation to mix the contents during processing.

***Exhausting*:** Exhausting aims to remove air from the package before closure.

***Heat processing*:** once cans are sealed they must be processed as soon as possible. Canning process involves packing the food and sterilizing both food and package at temperatures around 115 to 112°C for low acid foods (pH > 4.5) or at about 100°C for high acid foods (pH<4.5) (http:// www.simplot).

***Incubation and quality control checks*:** The controller/recorder maintains the temperature + 0.5°C throughout the process. Process temperature and the pressure during cooling are recorded on a 24-hour chart and kept as a permanent record.

2.3.2. Post Canning process

***Cooling*:** Water is used during the process to cool the containers after processing. The water used for cooling must be chlorinated to a level of 5mg/l. The chlorine is freed after 30 minutes contact time. Chlorination is essential as it reduces the chance that microorganisms will enter the can (many food spoilage losses have been attributed to this cause) (http:// www.simplot).

2.4 Microbiological significance of canning

The amount of heat delivered by a food process is dependent on the way the product is heated and its physical nature. Process-dependent factors can include processing equipment design, type

of heating media, container or food size and shape, product composition and viscosity (USFDA, 2000). The thermal destruction kinetics of microorganisms or their ability to be killed within the food matrix is likewise dependent on pH, a_w, level and type of preservatives, the previous growth conditions of the organism of concern, product composition and competitive microorganisms.

To make decisions on whether a food requires time/temperature control for safety, the properties of the food itself, added preservatives and processing steps, and the environmental circumstances that may affect their microbial ecology must be considered.

The microbiologist and food process engineer have to consider the viscosity of the carrier fluid, e.g. low thin soup, or medium gravy or high, such as concentrated starch. This fluid has a great effect on heat penetration into the product. The presence and the numbers of microorganisms in a product before processing will depend on the quality of the raw materials used and how they have been stored. This will be true for both the ingredients of the carrier fluid and any particulates; therefore the effective heat process must be designed to adequately reduce these organisms (Gaze, 2005).

In the canning industry, most food processing operations are designed to extend the shelf life of a product by eliminating undesirable microbial activity. The most recognized processing operation that involves the use of thermal energy is sterilization. It is the process of heating at a specified temperature/time to eliminate the pathogenic spores of concern from the product. This process is usually done using steam to heat the food in can (Abdul *et al.*, 1999).

The steam sterilization employs steam under pressure (15psi) to attain a temperature of 121°C, which is necessary to kill bacterial endospores (CFPRA, 1977 sited in Gonzalez *et. al.*, 2002). Both, a high temperature and sufficient time are required to be certain of adequate sterilization processing (Gonzalez *et al.*, 2002). This ensures that all parts of the food being canned have received enough heat to reduce the number of microorganisms to an extremely small and safe level with a long storage life.

Chemical and bio-chemical reactions in food materials during heating are temperature dependent. Such heating process not only destroys microorganisms but also destroys some of the valuable

nutrition. The complete destruction of microorganisms would result in a product with unacceptable quality and little nutritional value. So a best commercial process is that which has a maximum effect on spoilage organisms and minimal effect on quality of the nutritional value.

The major challenge to the thermally processed food manufacturer has, therefore, been to prepare food, which is safe in terms of the pathogenic organisms and microbiologically stable over its shelf life, but minimally processed to preserve good quality (Dewanto *et al.*, 2002).

Even though canned foods are considered to be commercially sterile, different studies have reported that canned foods contain microorganisms that cause food spoilage and poisoning. The microbial profile and potential hazards associated with imported and local brands of tomato paste in Nigeria indicated that four dominant bacterial genera (*Bacillus, Clostridium, Lactobacillus, and Leuconostoc*) were isolated and the greater proportions were spore formers (Efiuvwevwere and Atirike, 1998). Based on morphological and biochemical properties of a total of 50 strains of aerobic spore producing *Bacillus* isolated from semi canned meat, it was observed that the flora consisted of 50% *B. licheniformis*, 26% *B. subtilis*, 20% *B. pumilus*, and 4% *B. cereus* (Petrova, 1975). Another study using microtiter identification scheme, 115 strains of *Bacillus* spp were isolated from spoiled canned foods and most of the isolated strains belonged to the species *B. Subtilis, B. meganetarium* and *B. brevis* (Kotzekidou, 1996). Wojciechowski *et al.*, (1976) also found out that after the meat was contaminated with determined quantity of *B. subtilis* spores then after autoclave sterilization, thermal resistance *B. subtilis* spores were isolated from canned meat.

The case of food poisoning possibly caused by the ingestion of canned meat was also reported by Broek and Bijker (1976). Large numbers of microorganisms with a level of about 10^7/g, mainly *Enterobacteriaceae* and *Staphylococcus*, were isolated from the contents of three cans. The contents of another can had about 10^5/g *Bacillus* spp. The inadequate sterilization and errors in processing were suggested as possible causes (Broek and Bijker, 1976).

Can defect could also be source of canned food spoilage. The Alaska salmon industry conducted 9 recalls, which was implicated in illness and one death in Belgium from *Clostridium butolinum*

type E toxin. The salmon packer's plant had investigated that the equipment used to make cans was responsible for can defect of 300,000 cans examined (Haves, 1983).

Spoilage and pathogenic bacteria could also be possibly due to improper sterilization of cans. A study had confirmed that, from 1,393 canned fish products, which were kept at 37°C for 5-6 days and night, made it possible to detect all tins that were non sterile (Todorov, 1977). Another investigation had been carried out that a home canned tuna were reported in relation to a suspected botulism (Aureli *et al.*, 1984).

Four outbreaks of staphylococcal food poisoning in the United States were associated with eating mushrooms canned in the People's Republic of China (PRC) (Levine *et al.*, 1996). *Leuconostoc oenos* was also isolated from canned mango, which was the first report of *L. oenos* as a spoilage organism in fruit products other than wine (Ethiraj and Suresh, 1985).

Dried milks are considered sensitive products from a public health aspect because they are often consumed after reconstitution without additional heating. It is well known that dried milk can be a source of food borne illness because of contamination with *Salmonella, Staphylococcus*, and toxin production (Richter and Vedamuthu, 2001). Since the heat treatment is as mild as possible to maintain high nutritive quality, contamination of dried milks by *Salmonella* occasionally occurs, sometimes from the use of contaminated ingredients added during dry mixing or from the environment of the production line (Richter and Vedamuthu, 2001).

Many isolates of *Bacillus* spp are reported as human infectious agents. Opportunistic infections with *Bacillus* spp other than *B. anthracis* had been reported since the late 19th century (Kenneth, 2005).

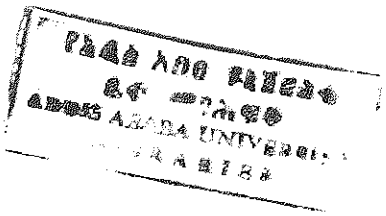
B. coagulans spores are commonly involved in the spoilage of canned foods known as flat-sour (Palop *et al.*, 1999). They are able to germinate and grow at pH values as low as 4, and are the microorganisms most frequently isolated from spoiled canned vegetables acidified to pH value between 4 and 4.5 (Mallidis *et. al.*, 1990). More over *B. coagulans* is able to increase the pH of

foods to values that can allow the germination of surviving butolinum spores (Fields *et al.*, 1977 and Anderson, 1984).

Bacillus cereus is aerobic spore former that is commonly found in soil, on vegetables and in many raw and processed foods. Consumption of foods that contain large numbers of *B. cereus* (10^6 or more /g) may result in food poisoning. *B. cereus* has long been associated with both food-borne illness and gastrointestinal infections. The latter infections are usually, but not always, opportunistic and are sometimes severe or life threatening (Bennett and Harman, 1988). The incrimination of *B. thuringiensis* in infection is rare but has occurred, while *B. mycoides* appears to be totally non-pathogen (Peter *et al.*, 2004).

Acidifications of some canned vegetables to pH lower than 4.5 before canning is a normal practice in food processing factories (Palop *et al.*, 1999). This practice has the advantage of reducing the thermal treatment of heat resistant microorganisms and preventing the out growth of spores surviving the heat treatment, especially spores of *C. butolinum* (Odlaug and Pflug, 1978).

Although there are various other species of *Bacillus*, which are regarded as saprophytic, a few, notable *B. pumilus* and to a lesser extent *B. subtilis* and *B. licheniformis* have been reported sporadically as pathogens (Karmer and Gilbert, 1989).



3. MATERIALS AND METHODS

A total of 100 samples of canned foods comprising 20 each of low acid products such as fish, meat, vegetable, milk and soups were collected from Addis Ababa supermarkets and grocer's shops randomly. Date of production and expiration, country of production and composition of ingredients of each sample were recorded. The samples were kept at room temperature until further analysis.

3.1 Examination and preparing of cans prior to opening

Before opening, cans were visually examined for presence of double seam and side seam defects, flaws and physical damage and pertinent observations were recorded. The non-coded end of the metal can was cleaned with alcohol-socked towel. This was flamed using a laboratory burner (Robert *et al.*, 1996).

This was then followed by shaking the cans to mix their contents. A sterilized opening device was used to cut the desired size entry hole. Under aseptic conditions, 25g of samples were removed from the centre and placed in to a sterile stomacher bag, 225 ml of sterile bacteriological peptone water (BPW) were added to it and homogenized for 2 minutes using a stomacher (Model 400, Seward) (Robert *et al.*, 1996). The homogenized samples were serially diluted.

3.2. Bacteriological count

3.2.1. Aerobic Mesophilic Count (AMC)

From appropriate dilutions, 0.1ml aliquots were spread-plated in duplicates on pre-dried surface of Plate Count (PC) agar (Oxoid) plates. Plates were incubated at 30-32°C for 24-72 hours for colony counting. Un-inoculated control plates were also incubated at the same temperature to check for sterility of plating media.

3.2.2 Counts of *Staphylococci*

From appropriate dilutions, 0.1ml aliquots were spread-plated in duplicates on pre-dried surface of Manitol Salt Agar (MSA) (Oxoid) plates and incubated at 30-32^{0}C for 24-72 hours. Golden yellow colonies were counted as staphylococci.

3.2.3 Coliform counts

From appropriate dilutions, 0.1ml aliquots were spread-plated in duplicates on pre-dried surface of Violet Red Bile Agar (VRBA) (Oxoid) plates. The plates were incubated at 32°C for 24 hours after which purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms.

3.2.4. Yeast counts

From appropriate dilutions, 0.1ml aliquots were spread-plated in duplicates on pre-dried surface of Chloramphenicol Bromophenol Blue Agar made from the following ingredients: (yeast extract 5g, glucose 10g, chloramphenicol 0.1g, Bromophenol blue 0.01g, agar 15g, distilled water 1000ml, pH, 6-6.4). The seeded culture plates were incubated at 32^{0}C for 3-5 days. The smooth (non-hairy) colonies without extension at periphery (margin) were quantified as yeast.

3.3. pH

The pH of each sample was determined from the original sample using pH meter.

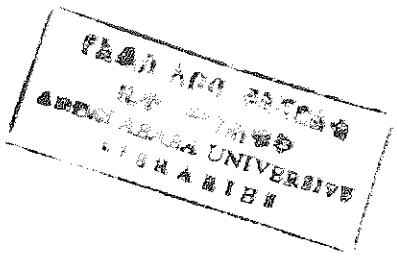
3.4. Flora analysis

After enumeration of aerobic mesophilic bacteria, about ten colonies were picked randomly from countable plates and separately inoculated into tubes containing 5ml of Nutrient Broth (Oxoid). These were incubated at 32°C overnight. Cultures were purified by repeated plating and were characterized to the genus level using the following tests.

3.4.1. Cell morphology

From an overnight pure plate culture, colonies were picked and mounted on microscopic slide. The preparation was observed under light microscope using oil immersion objective. An old culture was used for observing spores. The morphological criteria considered during the observation were:

Cell shape:	Regular: Rods, Coccoid forms
Cell arrangement:	Single, pairs, clusters, tetrads
Motility:	Motile, non-motile
Spore:	Presence, absence.



3.4.2. KOH test

This test was performed as proposed by Gregersen, (1978). One or two drops of 3% KOH solution were placed on a clean microscope slide. A colony was picked with a sterile bacteriological wire loop and stirred in the 3% KOH solution for 10 seconds to 2 minutes. The inoculating loop was raised slowly from the mass. When KOH solution became viscous, the thread of slime followed the loop for 0.5 to 2cm or more. Typically, this was observed in Gram negative bacteria. In case of no slime, watery suspension did not follow the loop, and this was seen in Gram-positive bacteria.

3.4.3. Oxidation-Fermentation (O/F) test

The utilization of glucose by each isolate was assessed by O/F test to identify microorganisms that metabolize glucose fermentatively or oxidatively or do not utilize glucose by either way.

Ingredients (g/l): peptone 2g, yeast extracts 5g, NaCl 5g, K_2HPO_4 0.3g, glucose 10g, agar 3g, distilled water 1000ml, bromothymol blue 0.08g, pH, 7.1.

The freshly prepared medium (15 ml amount in 18 x 180mm test tubes) was immediately cooled under tap water to prevent oxygen from dissolving into the medium and inoculated by stabbing

the culture with a sterile straight wire to the bottom. Acid formation and growth regions were interpreted after 2 and 5 days of incubation at 32°C.

3.4.4. Catalase test

This test was used to determine those organisms that produced catalase enzyme. The young colonies were flooded with a 3% solution of H_2O_2. The formation of bubble indicated the presence of catalase enzyme.

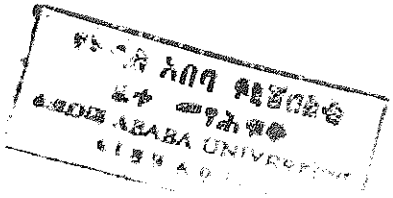
3.5. Isolation and characterization of *Salmonella* and *Shigella* spp

3.5.1 Primary enrichment

To test for these genera, 25g of samples were removed from the center and placed in to 225ml of sterile Buffered Peptone Water (BPW) and incubated at 32°C for 18-24 hours for the metabolic recovery and proliferation of cells in the food samples which could have been injured during canning process or to bring the number of target organisms to a detectable level.

3.5.2. Secondary enrichment

Secondary enrichment broths are essentially important in that they inhibit non-targeted microorganisms like Gram-positive bacteria and coliforms and permit the rapid multiplication of *Salmonella* and *Shigella* spp. Selenite Broth (SB) and Rappaport-Vassiliadis (RV) broth were employed for this enrichment purpose. After pre-enrichment in BPW, 1ml of culture was transformed into a tube containing 10ml of Selenite broth, and 0.1ml of BPW was also inoculated in to a tube containing 10ml of RV broth. SB broth was incubated at 37°C and RV broth at 43°C for 48 hours in water bath.



3.5.3. Isolation

Salmonella-Shigella (SS) Agar and Xylose Lysine Deoxycholate (XLD) medium (all from (Oxoid) were used for plating purposes. A loopful of culture from selective enrichment broth was streaked separately on to each of the solid media and incubated at 37°C for 18-24 hours.

3.6. Coagulase test for *Staphylococcus spp*

This test is used to detect the potentially pathogenic *Staphylococcus* spp, which is coagulase positive. Pure culture of *Staphylococcus* isolates were sub-cultured into 5ml of Nutrient Broth and incubated at 32°C for 24 hours. An overnight broth culture of *Staphylococcus* (0.5ml) was added to a tube containing 0.5 ml of sheep plasma. The tube was rotated gently to mix the contents and then incubated in water-bath at 37°C for 2-4 minutes or overnight for weak coagulase positive strains.

3.7. Identification and characterization of *Bacillus spp*

Isolates which were identified as *Bacillus spp from flora analysis* were transferred to 5ml of Nutrient Broth and further purified and preserved on Nutrient Agar slant for further identification.

3.7.1. Biochemical test for *Bacillus spp*

The following biochemical tests were employed for identification of the different *Bacillus* spp.

Oxidative-Fermentative (O/F) test
This test was conducted as indicated in **3.4.3.**

Growth at 50^0C and 65^0C
A loopful of pure culture was transferred to two tubes containing 5ml Nutrient Broth and the tubes were separately incubated at 50°C and 65°C. Growth of culture was noted after 3 days and 5 days, respectively.

Growth in 7% NaCl

A loopful of pure culture was transferred to Nutrient Broth tubes containing 7% NaCl. Growths of culture were observed from 7 to 14 days after incubation at 32°C.

Hydrolysis of starch

This test is used to differentiate bacteria based on their ability to hydrolyze starch using the exoenzyme amylase. Soluble starch (6g) was dissolved in 60ml of distilled water and steamed for 10min. Melted sterile Nutrient agar (Oxoid) was mixed with soluble starch aseptically in proportion of 20ml starch in to 100ml of NA.

Gram's iodine solution was prepared by dissolving 2g potassium iodide in 20ml of distilled water and 1g of finely ground iodine was added. The solution was allowed to stand overnight. Distilled water was added to make up to 300ml.

A loopful of culture was inoculated along the diameter of the starch agar plate and incubated at 32°C. After incubation for 3 and 5 days, the growth was flooded with iodine solution. Formation of clear zone around growth indicated utilization of starch.

Voges Proskauer Reaction (VP)

The VP test detects organisms that use the butylene glycol pathway and produce acetoin. When the VP reagents are added to MR-VP broth that has been inoculated with an organism that uses the butylene glycol pathway, the acetoin end product is oxidized in the presence of potassium hydroxide (KOH) to diacetyl. Creatine is also present in the reagent as a catalyst. Diacetyl then reacts to produce a red color.

A loopful of culture was inoculated in to 5ml of MR-VP broth. The culture was incubated at 32°C for 3-5 days. Three ml of 5% alcoholic α-naphtol solution (5g-α-naphtol in 100ml of absolute alcohol) and three ml of 40 % (w/v) potassium hydroxide (KOH) were added into the culture. The mixture was shaken gently. Bright red colour was recorded as positive test.

Citrate test

The citrate test is used to determine the ability of a bacterium to utilize citrate as its sole source of carbon. Bacteria can break the conjugate base salt of citrate into organic acid and carbon dioxide. The carbon dioxide can combine with the sodium from the conjugate base salt to form a basic compound sodium carbonate. A pH indicator in the medium detects the presence of this compound by turning blue.

A sterilized citrate agar was slanted in a test tube. Using sterilized needle the slant was streaked with the culture. The inoculated culture was incubated at 37°C for 7 to 14 days. Formation of blue colour on slant indicated utilization of citrates.

3.8. Antimicrobial susceptibility testing for *Bacillus* spp

Antimicrobial susceptibility testing was done for 256 isolates. The slanted cultures were sub-cultured and purified. From Nutrient Agar plates, two to three pure colonies were inoculated in to Nutrient Broth and incubated at 32°C for 18-24 hours. The growth was standardized to optical density of 0.5 McFarland Standard to bring the cell density to about 10^7-10^8cfu/ml. The McFarland turbidity standard was prepared by mixing 0.1 ml $BaCl_2$ (1%) with 9.9ml H_2SO_4 (1%) (Jorgenson *et al.*, 1999). Muller Hinton (MH) (Oxoid) plates were prepared and warmed to room temperature for plating. A sterile cotton swab was dipped in to the standardized suspension and the excess was removed by turning the swab against the side of the container. The cultures were spread evenly over the entire surface of the Muller Hinton Agar plate by swabbing in three directions at 90° of each spreading .The plates were allowed to dry before applying antimicrobial discs.

The following antibiotic discs (all from Oxoid) were applied in this study: Erythromycin (Ery) (15µg), Kanamycin (Kan) (30µg), Sulfamethoxazole-Trimethoprim (Sxt) (25µg), Amoxycillin (Aml) (2µg), Ampicillin (Amp) (10µg), Gentamycin (Gen) (10µg), Streptomycin (Str) (10µg), Methicilin (Met) (5µg), Chloramphenicol (Chl) (30µg), Tetracycline (Tet) (30µg), Vancomycin (Van), (30µg), Penicillin G (Pen G) (l0iu), Polymyxin B (Pol B) (30 iu) and Cephalothin (Cep) (30µg).

The discs were dispensed on to the surface of Muller Hinton agar plates using a dispenser. The discs were firmly applied to the surface of an agar plate, which was previously dried. The contact with the agar was even. Plates were incubated at 32°C for 18-20 hours. Diameter of zones of inhibition was measured by using a ruler in mm. The measurement was translated in to descriptive terms as susceptible (S), or resistant (R) according to off-points given by National Committee for Clinical Laboratory Standard (NCCLS, 1998 cited in Jorgenson *et al.*, 1999). All intermediate results were considered sensitive for the purpose of interpretation.

3.9. Determination of Growth Potential of pathogens in canned foods

This study was made to asses the potential hazards related to possible kitchen contaminations after cans are opened and left-overs are kept for later consumption. The growth potential of Salmonella Typhimurium and Shigella flexineri was assessed in canned Tuna, Farm Style Vegetable, Asparagus, Creamy Chicken Soup, and Borena Corned Beef. Two cans of each food item were opened aseptically and separately inoculated with overnight culture of the test strains to give an initial inoculum level of 10^2-10^3 cfu/ml. The inoculated foods were mixed thoroughly aseptically and incubated at ambient (20-25°C) and refrigeration (4°C) temperature for 48 hours and 5 days, respectively. To determine the initial inoculum level, freshly inoculated foods (10g each) were homogenized separately into 90 ml of BPW and 0.1 ml of appropriate dilutions were spread plated on McConkey Agar (Oxoid). Portions of inoculated food items (10g each) were further sampled aseptically at six-hour intervals for 48 hours for ambient temperature and at one-day intervals for five days for refrigeration temperature.

4. RESULTS

Various types of canned foods are imported to Ethiopia in different brand forms. Limited types of canned foods are also produced locally. Supermarkets and grocer's shops are the major outlets where the canned products are made available to the consumer. Some canned foods are sold only in super markets while others such as Sardines, Tuna, Nan, Nido and others are available in both supermarkets and grocer's shops.

Venders kept all canned foods at room temperature. Each canned food was labeled with date of production and expiration, ingredients, and appropriate condition of storage etc. In some canned foods date of production was not given and most do not give any information about canning processes.

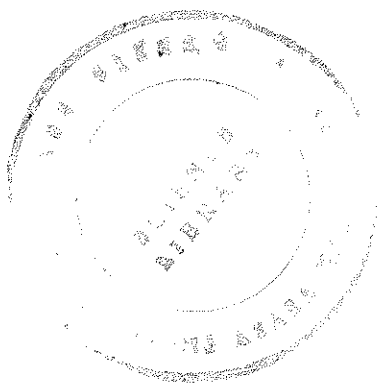
4.1. Description of canned foods

4.1.1. Canned fish products

Both Sardine and Tuna are fish products. Ingredients were sunflower oil (Vegetable oil) fish and salt. Tuna was minced fish meat while Sardine was made from whole small fish of which the tail, head, visceral organs and bones were removed. Date of expiration and production was labeled.

4.1.2. Canned meat products

These products consisted of Borena Corned Beef and Beef-in-Jelly. Borena Corned Beef was prepared by cooking in salty water and then pressing it into metal containers where it could be kept for long time. It was produced in Ethiopia. Its ingredients were corned beef, salt, sodium nitrate and spices. Date of production of this product was not labeled. Beef-in-Jelly was a meat product impregnated with jelly. The ingredients were beef, jelly, salt and spice. It was produced in Ethiopia. Date of production was not labeled.



4.1.3. Canned vegetables products

Vegetable products consisted of canned Asparagus, Farm Style Vegetable and Mixed Vegetables. Asparagus was prepared as spears peeled white. Ingredients were Asparagus, water and salts. Date of production was not labeled on the South African products while products of China were properly labeled.

Farm Style Vegetable product was produced in U.S.A. The ingredients were water, tomatoes, carrots, potatoes, processed peas, butter, cornstarch, barely, salt, cane sugar, vermicelli, onions and spices. Only the expiration date of the product was labeled. Cooking instruction was stated on the label. Refrigeration of unused soup was recommended.

Ingredients of Mixed Vegetable were water, carrot, potatoes, celery, sweat peas, green beans, corn, lima beans, salt, onion, and flavoring calcium chloride. The contents were recommended to be consumed without further cooking. Date of production and expiration was labeled. These were products of South Africa and U.S.A.

4.1.4. Canned soup products

These consisted of two product types, namely Creamy Chicken Soup and Beef and Vegetable Soup. Creamy Chicken Soup was a condensed soup product. Ingredients were chicken stock, chicken thickener (modified maize starch), skim milk, wheat, flour, cream, salt, flavors, sugar, spices, Natural colour (β- carotene, Riboflavin) and water. It had no preservatives and artificial flavors. Cooking guides were given on the label. Boiling was not recommended. Products were made in Australia, England and the Netherlands. All had date of expiry but products from England did not have date of production.

The contents of Beef and Vegetable Soup were water, tomatoes, carrots, beef, beans, barley, cornstarch, butter, peas, salt, and onions, flavoring agents, cane sugar and spice. Cooking instructions were given on label. Boiling was not recommended. Unused soup was suggested to be refrigerated properly. Date of expiration was given on label but not date of production. It was a U.S.A. product.

4.1.5. Canned powdered milk products

The common canned powdered milk products in supermarkets and grocer's shops were Nido and Nan. Nido is infant formula milk. Average nutritional information per 1000g of powder was given on label. Preparation instructions were given on label.

Nan is also an infant formula, which is recommended for infants who are not being breastfed. It was made of cow's milk and various other nutritional additives. Both milk products were made in Netherlands and Dubai. Date of production and expiration of both products was labeled.

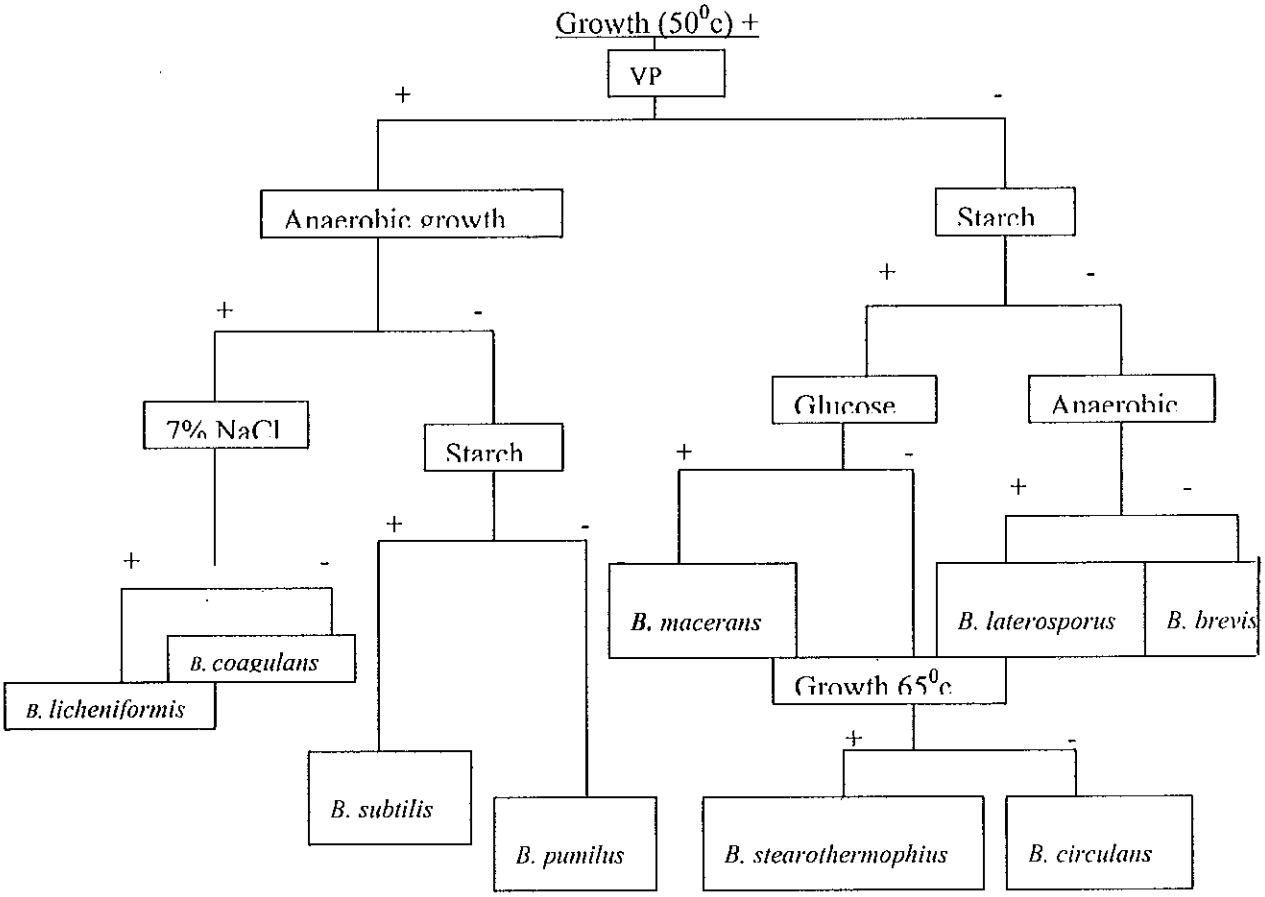
4.2. Examination of cans

Cans were visually examined and none of the 100 samples had any defect of physical nature and seams were normal.

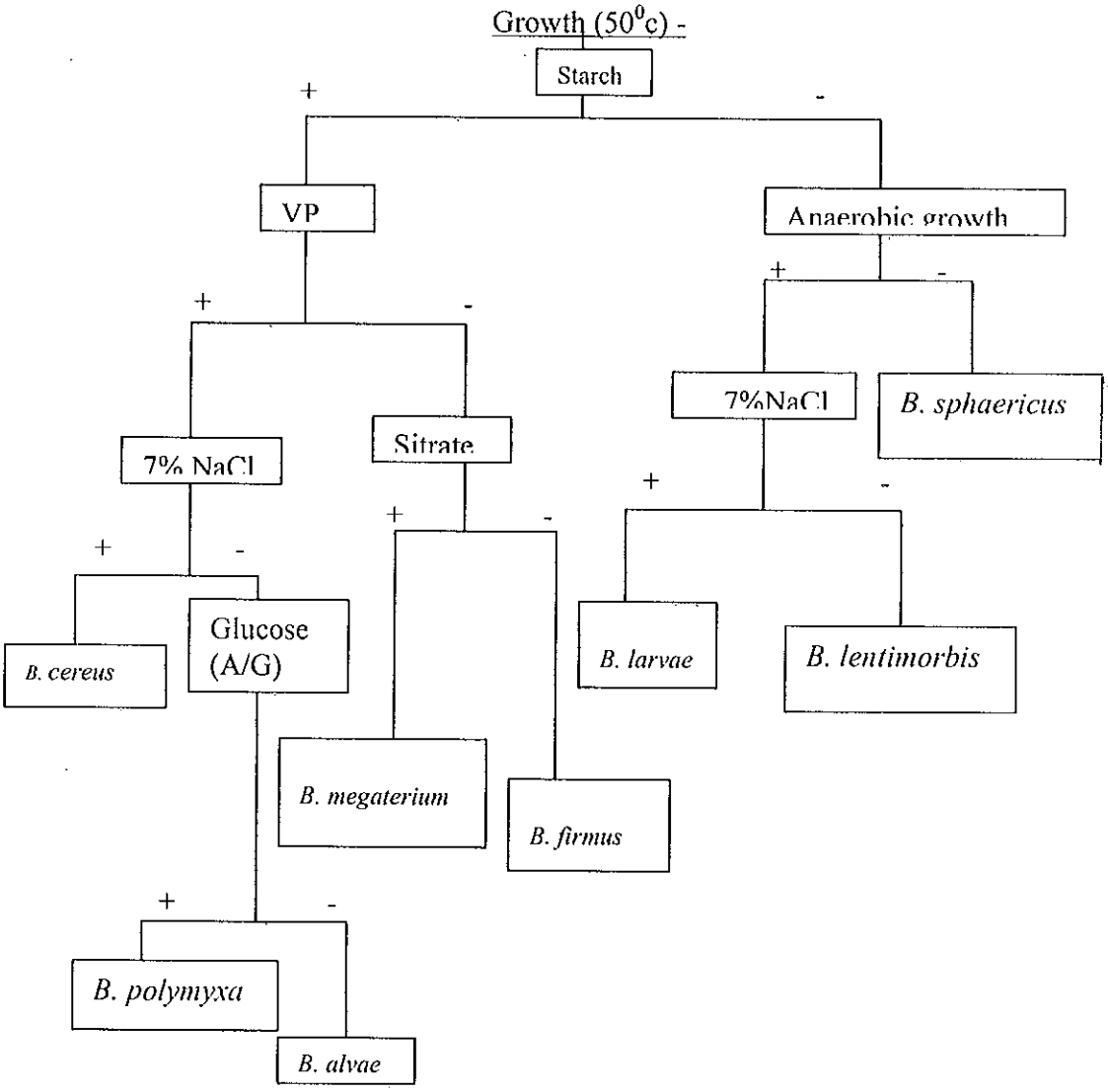
4.3. Microbial spectrums of canned foods

A total of 679 bacterial isolates were isolated from 93 canned foods and were characterized to various genera and bacterial groups. Different genera constituted the dominant microflora. The aerobic microflora was dominated by *Micrococcus* spp (40.3%), *Bacillus* spp (37.7%) and other Grampositive Rods (18.3%). Out of 100 samples, 7 (2 Farm style vegetable, 1 Nan, 4 Vegetable and beef soups) had microbial load below detectable levels by the methods used in this study < 2 (log cfu/g).

A total of two hundred fifty six *Bacillus* species, isolated from the various types of canned foods, were identified to the species level using the simplified identification scheme of Berkeley *et al.*, (1984).). If isolates were positive for growth at 50^0c method 1 used, method 2 otherwise. A total of 17 species were identified among them *B. cereus, B. firmus, B. licheniformis, B. brevis*, B. *macerans, B. larvae, B. subtilis, B. pumilus and B. circulans*, were the most common isolates.



Method 1, Identification of *Bacillus* species Berkeley *et al.*, (1984)



Method 2, Identification of *Bacillus* species Berkeley *et al.*, (1984).

4.3.1. Canned fish products

The mean aerobic mesophilic counts in each product item of Sardine and Tuna were around 4.22 and 4.14 (log cfu/g), respectively. Variations in counts within each product type in either of the products were significant (CV>10%) (Table 1). The mean pH value of Sardine and Tuna was 5.84 and 5.72, respectively (Table 1). *Staphylococcus* spp was encountered in 2 samples of Sardines and 4 samples of Tuna. Higher counts were seen on Tuna (data not given) (log cfu/g). Counts of coliforms, *Staphylococcus* and yeasts were comparable between Sardines and Tuna (Table 2).

The aerobic mesophilic counts of Sardine were dominated by *Bacillus* spp (61.8%), other Gram positive rods (25%) and *Micrococcus spp* (13.2%), whereas those of Tuna were dominated by *Micrococcus* spp (52.3%) and *Bacillus* spp (43.1%) (Table 3). Among the *Bacillus* isolates *B. firmus, Bacillus cereus* and *B. licheniformis* were dominant in both Sardines and Tuna (Table 4).

Table 1. Mean counts (log cfu/g) of aerobic mesophilic bacteria of canned food samples

Group	Sample type	pH Mean ± SD	No of samples	Aerobic mesophilic bacteria (log cfu/g)		
				Mean ± SD	SD	%CV
Fish	Tuna	5.72 ± 0.11	10	4.14 ± 0.54	0.54	13%
	Sardine	5.84 ± 0.13	10	4.22 ± 0.64	0.64	15%
Meat	Borena Corned Beef	5.90 ± 0.04	10	3.68 ± 0.38	0.38	10%
	Beef in Jelly	5.70 ± 0.11	10	3.86 ± 0.53	0.53	14%
Vegetable	Mixed Vegetable	5.23 ± 0.29	6	4.91 ± 1.32	1.32	27%
	Asparagus	5.26 ± 0.26	10	3.47 ± 1.05	1.05	30%
	Farm style Vegetable	5.62 ± 0.01	4	5.56 ± 0.03	0.03	0.60%
Soup	Creamy chicken Soup	5.26 ± 0.29	10	4.24 ± 0.55	0.55	13%
	Beef and Vegetable soup	4.68 ± 0.02	10	3.75± 0.39	0.39	10%
Powdered milk	Nan	6.41 ± 0.01	10	3.25 ± 0.56	0.56	17%
	Nido	6.17 ± 0.02	10	2.79 ± 0.23	0.23	8%

4.3.2. Canned meat products

All 10 samples of Beef-in-Jelly and Borena Corned Beef had mean microbial count of 3.86 (log cfu/g) and 3.68 (log cfu/g), respectively (Table 1). The maximum counts reached 4.48 for corned beef and 4.61 for Beef-in-Jelly (data not available). The mean pH value of Borena Corned Beef and Beef-in-Jelly was 5.90 and 5.70, respectively (Table 1). Variation in microbial counts within samples of Beef in Jelly was significant (CV= 14%) (Table 1). The flora in both canned meat products was dominated by *Bacillus* spp, *Micrococcus* spp and other Gram positive rods. *Bacillus* spp made (53.8%) of the dominant flora in Borena Corned Beef, while in Beef-in-Jelly they constituted 44.3% of the dominant flora (Table 3).

Staphylococcus spp and coliforms were encountered only in one each of Borena Corned Beef and Beef-in Jelly samples, respectively at very low levels 2 (log cfu/g). Yeasts were not found in any of the canned beef products (Table 2). *B. cereus, B. licheniformis, B. macerans, B. circulans* and *B. subtilis* were the dominant isolates of both canned meat products (Table 4).

Table 2: Counts Coliforms, *Staphylococcus, and* Yeasts in some canned food samples

Samples type	Counts > 2 log (cfu/g)					
	Coliforms		*Staphylococcus*		Yeast	
	No samples	Mean ± SD	No samples	Mean ± SD	No samples	Mean ± SD
Tuna	1	2.3	4	2.75 ± 1.08	1	2
Sardine	3	2.73 ± 0.67	2	2.54 ± 0	-	-
Beef-in-Jelly	1	2.18	-	-	-	-
Borena Corned Beef	-	-	1	2.18	-	-
Creamy Chicken Soup	-	-	2	2.83 ± 0	-	-

NB counts of Coliforms, *Staphylococcus* and yeast in other canned foods products were below detectable level or nil.

4.3.3.Canned vegetable products

The mean microbial counts of Mixed Vegetables, Asparagus and Farm Style Vegetables was 4.91, 3.47 and 5.56 (log cfu/g), respectively (Table 1). Significant variation was observed in counts within samples of Asparagus and Mixed Vegetables (CV>25%).

Counts of coliforms, *Staphylococcus* spp and yeasts were below detectable levels<2 (log cfu/g) in all canned vegetable products. The mean pH value of Asparagus, Mixed Vegetables and Farm Style Vegetables were ranged between 5.23 and 5.62 (Table 1)

The aerobic microflora of the three types of canned vegetable products was dominated by *Micrococcus* spp followed by *Bacillus* spp Other Gram positive rods also constituted a minor proportion of the dominant flora in all samples (Table 3).

B. cereus, B. brevis, B. larvae, B. pumilus, B. licheniformis and B subtilis were dominant isolates of *Bacillus spp* in three canned vegetable products (Table 4).

Table 3: Frequency distribution of dominant bacteria in various canned foods

Groups	Sample[1]	No isolates	Number (%) of isolates				
			Bacillus spp	*Micrococcus* spp	*Streptococcus* spp	*Staphylococcus* spp	OGPR[2]
Fish	TU	65	28(43.1)	34(52.3)	2(3.1)	-	1(1.5)
	SR	68	42(61.8)	9(13.2)	-	-	17(25.0)
Meat	BCB	65	35(53.8)	16(24.6)	-	4(6.2)	10(15.4)
	BJ	61	27(44.3)	16(26.2)	-	2(3.3)	16(26.2)
Vegetables	MV	48	7(14.6)	30(62.5)	-	6(12.5)	5(10.4)
	AS	73	32(43.8)	33(45.2)	-	-	8(10.9)
	FSV	17	6(35.3)	8(47.1)	2(11.8)	-	1(5.9)
Soup	CCS	80	28(35.0)	36(45.0)	2(2.5)	-	14(17.5)
	VBS	49	14(28.6)	22(44.9)	4(8.2)	-	9(18.4)
Powdered Milk	NN	73	17(23.3)	35(47.9)	1(1.4)	-	20(27.4)
	ND	80	20(25.0)	35(43.7)	2(2.5)	-	23(28.7)
Total		679	256(37.7)	274(40.3)	13(1.9)	12(1.8)	124(18.3)

[1]AS = Asparagus, BJ = Beef in Jelly, BCB = Borena Corned Beef, CCS = Creamy Chicken Soup, FSV = Farm Style Vegetable, MV = Mixed Vegetable, ND = Nido, NN = Nan, SR = Sardines, TU = Tuna, VBS =Vegetable & Beef Soup [2] OGPR = Other Gram Positive Rods

4.3.4. Canned soup products

All samples of Creamy Chicken Soup and 6 samples of Beef and Vegetable Soup had mean microbial count of 4.24 (log cfu/g) and 3.75 (log cfu/g), respectively (Table 1). The mean pH

value of Creamy Chicken Soup and Beef and Vegetable Soup was from 5.26 and 4.68, respectively (Table1).

Significant variation was observed in microbial counts within samples of Creamy Chicken Soup (CV=13%) (Table 1). Counts of coliforms, *Staphylococcus* spp and yeasts were either below detectable level<2 (log cfu/g) or very low in both products (Table 2).

The aerobic microflora of Creamy Chicken Soup and Beef and Vegetable Soup was dominated by *Micrococcus* spp and *Bacillus* spp (Table 3). Among the isolates of the *Bacillus* spp from the canned soup products *B. brevis*, *B. cereus* and *B macerans* were the dominant isolates (Table 4).

Table 4: Distribution of the dominant isolates of *Bacillus* in canned food products

	Canned food items					
Isolates	Vegetables	Beef	Soup	Fish	Milk	Total
B. cereus	8	12	8	10	5	43
B. firmus	0	6	5	23	1	35
B. licheniformis	5	11	2	10	2	30
B. brevis	7	1	10	1	5	24
B. macerans	1	9	8	3	2	23
B. larvae	6	2	2	7	4	21
B. subtilis	5	7	1	4	2	19
B. pumilus	6	2	3	2	4	17
B. circulans	3	9	0	0	3	15
Other Bacillus spp	4	3	3	10	9	29

4.3.5. Canned powdered milk products

The aerobic mesophilic microbial load of Nan and Nido had mean microbial count of 3.25 (log cfu/g) and 2.79 (log cfu/g) respectively (Table 1). Significant variation was observed in counts within Nan samples (CV=17%) (Table 1). The mean pH value of Nido and Nan were 6.17 and 6.41, respectively (Table 1).

The aerobic microflora of both powdered milk products was dominated by *Micrococcus* spp and *Bacillus* spp (Table 3). Counts of coliforms, *Staphylococcus* spp and yeasts were below detectable level <2 (log cfu/g). *B. cereus* and *B. brevis* dominated the *Bacillus* isolates in both canned milk product types (Table 4).

Salmonella spp and *Shigella* spp were not isolated from all samples of canned foods. None of the *Staphylococcus* isolates produced the enzyme coagulase.

4.6. Antibiotic resistance pattern of *Bacillus* spp, isolated from various canned foods

A total of 256 *Bacillus* species, isolated from the different canned products, were tested for their antimicrobial sensitivity. Among these, *B. cereus, B. firmus* and *B. licheniformis* were the dominant species. Of the 43 isolates of *B. cereus,* more than 50% were resistant to Amoxycillin, Ampicillin, Cephalothin, Methicillin, Penicillin G, Sulfamethoxazole-Trimethoprim and Tetracycline. All of the isolates were susceptible to Erythromycin, Vancomycin and Kanamycin (Table 5). Thirty two isolates showed seven different patterns of multi-drug resistance including resistance to nine different drugs. The commonest pattern was Aml/Amp/Cep/Met/PenG/Sxt/Tet (Table 6).

Over 50% of *B. firmus* were resistant to Amoxycillin, Ampicillin, Cephalothin, Methicillin, Penicillin G and Sulfamethoxazole-Trimethoprim. All of these isolates were susceptible to Erythromycin, Kanamycin and Vancomycin (Table 5). Seventeen isolates of this species showed different multi-drug resistance patterns for more than four antibiotics. The commonest pattern was Aml/Amp/Cep/Met/PenG/PolB/Sxt/Tet (Table 6).

Table 5. Antibiotic resistance of the dominant *Bacillus* isolates from various canned foods

Identification	Total strains	Aml		Amp		Cep		Chl		Ert		Gen		Kan		Met		PenG		PolB		Str		SXT		Tet		Van	
		R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
B. brevis	24	14	10	19	5	3	21	1	23	-	24	-	24	-	24	5	19	7	17	3	21	-	24	4	20	6	18	-	24
B. cereus	43	37	6	33	10	24	19	1	42	-	43	7	36	-	43	27	16	38	5	12	31	1	42	28	15	22	21	1	42
B. circulans	15	7	8	8	7	6	9	1	14	-	15	1	14	-	15	6	9	9	6	2	13	1	14	3	12	5	10	-	15
B. firmus	35	31	4	34	1	15	20	1	34	-	35	-	35	-	35	14	21	16	19	7	28	1	34	19	16	11	24	-	35
B. larvae	21	8	13	9	12	8	13	1	20	1	20	-	21	2	19	6	15	9	12	2	19	2	19	4	17	7	14	2	19
B. licheniformis	30	16	14	15	15	15	15	2	28	1	29	-	30	2	28	12	18	15	15	2	28	1	29	9	21	12	18	1	29
B. macerans	23	15	8	16	7	8	15	3	20	1	22	-	23	-	23	15	8	16	7	5	18	-	23	15	8	15	8	1	22
B. pumilus	17	6	11	9	8	5	12	-	17	-	17	-	17	-	17	5	17	8	9	2	15	-	17	-	17	7	10	-	17
B. subtilis	19	10	9	9	10	9	10	1	18	-	19	1	18	-	19	6	13	10	9	6	13	1	18	5	14	6	13	-	19

Nearly half of the 30 isolates of *B. licheniformis* were resistant to Amoxycillin, Ampicillin, Cephalothin, Methicillin, Penicillin G and Tetracycline (Table 5). However, almost all were sensitive to Chloramphenicol, Erythromycin, Gentamycin, Kanamycin, Polymyxin B, Streptomycin and Vancomycin (Table 5). Almost half of isolates of this species were multidrug resistant. Three patterns of multi-drug resistance were observed among this species and the commonest pattern was Aml/Amp/Cep/Met/PenG/Sxt/Tet (Table 6). Except 2 of *B. alvae* and 1 each of *B. cereus, B. licheniformis* and *B. macerans* were resistant against vancomycin, 2 each of *B larvae* and *B. licheniformis* against kanamycin, and 1 each of *B. larvae*, *B. licheniformis* and *B. macerans* against erythromycin (Table 5).

Most of the other *Bacillus* species were sensitive to Chloramphenicol, Erythromycin, Gentamycin, Kanamycin, Streptomycin and Vancomycin. Resistance to Amoxycillin, Ampicillin, Penicillin G and Tetracycline was common among these species. Multiple resistances to Amoxycillin, Ampicillin, Cephalothin and Methicillin was very common in all the other species.

Table 6: Multi-drug resistance pattern of *Bacillus* isolates for from various canned foods

Strain	Multi drug resistance strains	Resistance pattern (autobiogram)	No of strains resistance for > 4 drugs
B. alvae	2	Aml, Amp, Cep, Met, Sxt, Tet	1
		Aml, Amp, Cep, Met, PenG, PolB, Sxt, Tet	1
B. brevis	6	Aml, Amp, Cep, Met, PenG, PolB, Sxt, Tet	1
		Aml, Amp, PenG, PolB, Sxt, Tet	2
		Aml, Amp, Cep, PenG, Sxt, Tet	1
B. cereus	32	Aml, Amp, Cep, Gen, Met, PenG, PolB, Sxt, Tet	2
		Aml, Amp, Cep, Gen, Men, PenG Sxt, Tet	2
		Aml, Amp, Cep, Met, PenG, PolB, Sxt, Tet	1
		Aml, Amp, Cep, Met, PenG, Sxt, Tet	6
		Aml, Amp, Cep, Met, PenG, PolB, Sxt	2
		Aml, Amp, Cep, Met, PenG, Sxt	3
		Aml, Amp, Cep, PenG, Sxt	2
B. circulans	8	Aml, Amp, Cep, Gen, Met, PenG, Sxt	1
		Aml, Amp, Cep, Met, PenG, Sxt, Tet	1
		Aml, Amp, Cep, Chl, Met, PenG, Tet	2
		Aml, Amp, Cep, Met, PenG, Tet	1
B. firmus	24	Aml, Amp, Cep, Met, PenG, PolB, Sxt, Tet	3
		Aml, Amp, Cep, Met, PenG, PolB, Sxt	2
		Aml, Amp, Cep, Chl, PenG, Sxt, Tet	1
		Aml, Amp, Cep, Met, PenG, Sxt, Tet	2
		Aml, Amp, Cep, Met, PenG, Sxt, Str	1
		Aml, Amp, Cep, PenG, PolB, Sxt, Tet	1
		Aml, Amp, Cep, PenG, Sxt, Tet	2
		Aml, Amp, Cep, Met, PenG, Sxt	2
		Aml, Amp, Cep, PenG, Sxt	3
B. larvae	9	Aml, Amp, Cep, Met, PenG, Str, Sxt, Tet	1
		Aml, Amp, Cep, Met, PenG, PolB, Sxt, Tet	1
		Aml, Chl, Kan, Met, PenG, Str, Van	1
		Aml, Amp, Cep, Ert, PenG, Sxt, Van	1
		Aml, Amp, Cep, Met, PenG, Tet	2
		Aml, Amp, Cep, PenG, PolB, Tet	1
B. lentimorbis	2	Aml, Amp, Cep, Met, PenG, Sxt, Tet	1
		Aml, Amp, Cep, Met, PenG, Sxt,	1
B. licheniformis	16	Aml, Amp, Cep, Kan, Met, PenG, PolB, Sxt, Tet	1
		Aml, Amp, Cep, Met, PenG, Sxt, Tet	3
		Aml, Amp, Cep, Met, PenG, Sxt	2
		Aml, Amp, Cep, Met, PenG, Tet	3
B. macerans	16	Aml, Amp, Cep, Met, PenG, PolB, Sxt, Tet, Van	1
		Aml, Amp, Cep, Met, PenG, PolB, Sxt, Tet	2
		Aml, Amp, Cep, Met, PenG, Sxt, Tet	1
		Aml, Amp, Met, PenG, Sxt, Tet	6
		Aml, Amp, Cep, Met, PenG, Sxt	2
B. polymixa	3	Aml, Amp, Cep, Met, PenG, PolB, Sxt	1
		Aml, Amp, Cep, Met, PenG, Sxt	1
B. pumilus	5	Aml, Amp, Cep, Met, PenG, Tet	5
B. sphaericus	3	Aml, Amp, Cep, Met, PenG, PolB, Str, Sxt, Tet	2
		Aml, Amp, Cep, Met, PenG, PolB, Tet	1
B. subtilis	8	Aml, Amp, Cep, Met, PenG, Sxt, Tet	2
		Aml, Amp, Cep, Met, PenG, PolB, Sxt	2
		Aml, Amp, PenG, PolB, Tet	2

4.7. Assessment of growth potential of test strains in some canned foods

At room temperature storage, the growth of *Shigella flexineri* increased by 1 log unit in all canned food products within 6 h of inoculation. Its growth markedly increased between 6 and 12 h in Tuna, Asparagus and Creamy Chicken Soup. The fastest growth rate of the test strain was seen in all canned food products between 12 and 24 h. Maximum counts of 9-log cfu/g were reached at 24 h in Borena Corned Beef and Asparagus. In the other canned foods, the test strain reached highest counts at 36 h. (Fig 1).

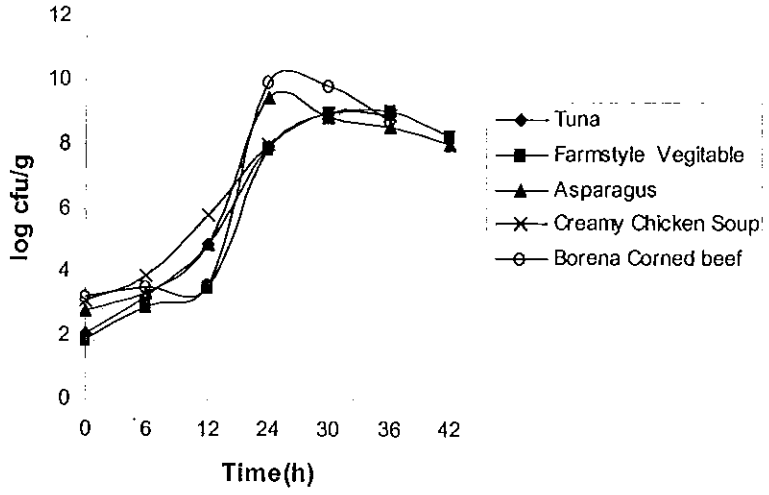


Fig 1. Growth pattern of Shigella flexineri at room temperature in five canned food products.

When stored at room temperature, *Salmonella* Typhimurium increased by about 1 log unit in the first 6 hours and then showed a steady growth up to 12 h in all canned foods. Its growth rate in Farm Style Vegetable and Creamy Chicken Soup was relatively higher than in the other canned food products. In most of the canned food products, the highest counts > 8 (log cfu/g) were

reached at 24 hours. In Creamy Chicken Soup and Farm Style Vegetable, however, the highest count was reached at 30h. The *Salmonella* test strain still had counts as high as log 8 cfu/g after 36 hours of storage (Fig 2).

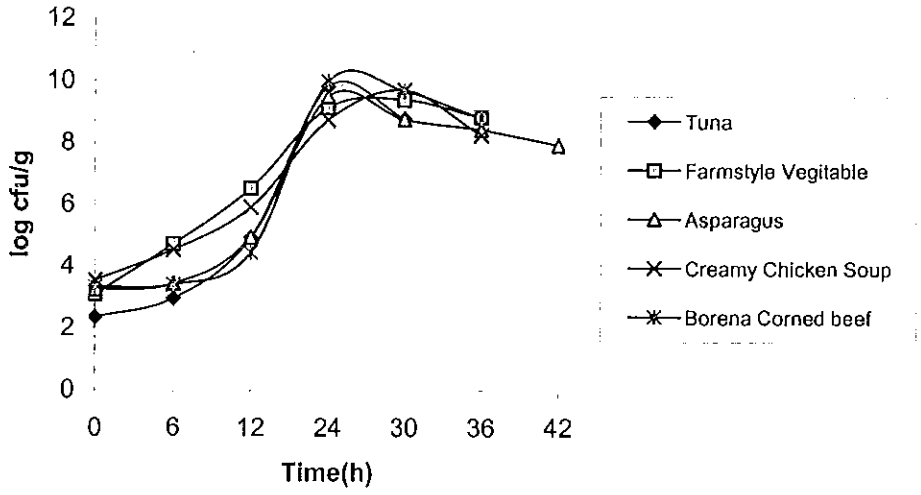


Fig 2. Growth pattern of *Salmonella* Typhimurium at room temperature in five canned food products

At refrigeration temperature storage, *Shigella flexineri* showed growth starting from day 1 in Tuna, Farm Style Vegetable and Asparagus. On the other hand, growth was not detected until day 3 in refrigerated Creamy Chicken Soup and Borena Corned Beef. Growth after 3 days was steady in all cases and highest numbers were reached at day 5 $\geq$ 7 (log cfu/g) (Fig 3).

At refrigeration temperature, *Salmonella* Typhimurium grew steadily in Farm Style Vegetable from day 1. In the other products, growth was markedly low until day 2. Growth was steady in all cases thereafter with final count reaching > 7 log cfu/g (Fig 4).

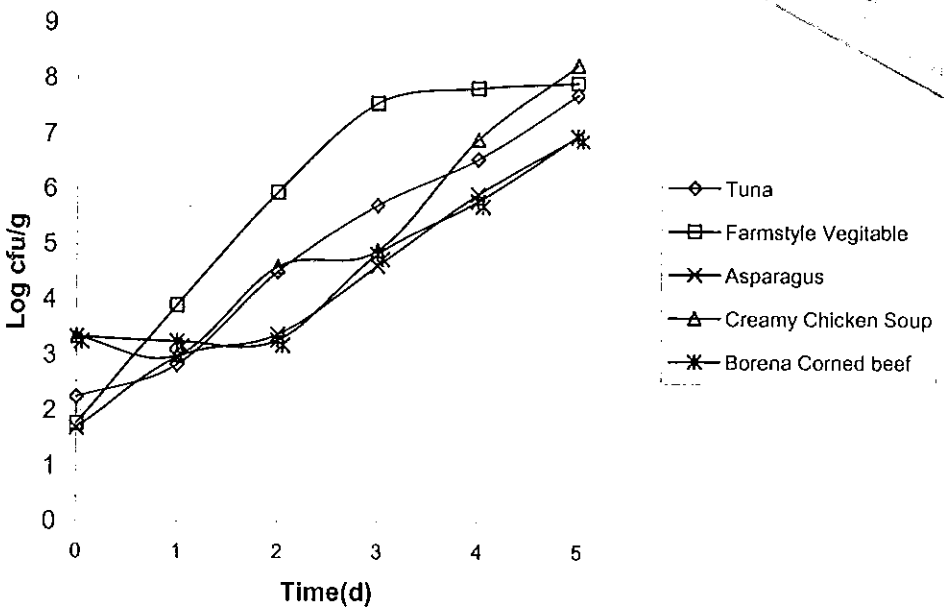


Fig 3.Growth pattern of *Shigella flexineri* at refrigeration temperature in five canned food products

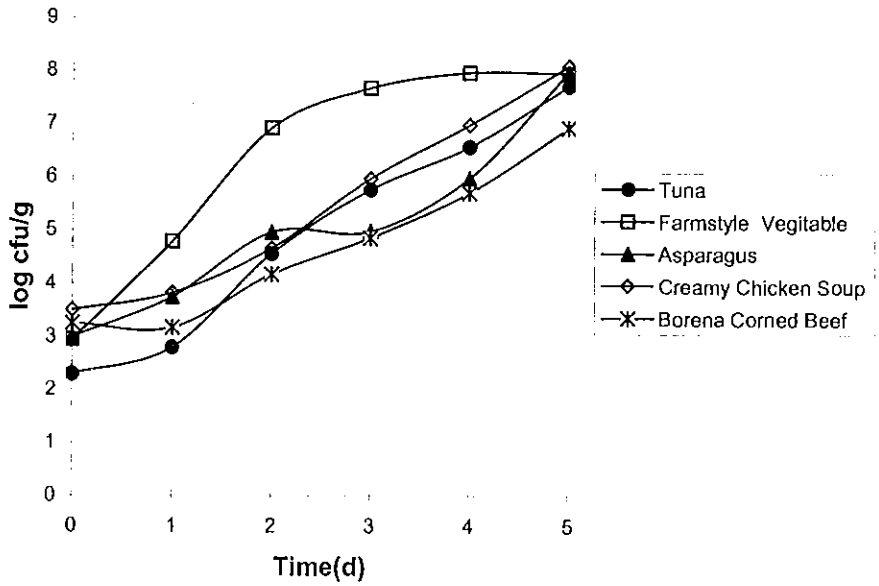


Fig 4. Growth pattern of *Salmonella* Typhimurium at refrigeration temperature in five canned food products.

5. DISCUSSION

Canning aims to have a product, which is free from pathogens and other microorganisms, which might spoil the food in the container. Canned foods are considered to be commercially sterile, i.e. the thermal treatment is necessary to render the food product free of viable microorganisms having public health significance as well as microorganisms with no health significance.

Despite the above facts, canned foods can be subjected to microbial spoilage due to various reasons. Spoilage of canned foods is usually caused by growth of microorganisms following leakage or under processing.

This study was conducted on various canned foods that were imported and locally produced canned food items. They were purchased from different supermarkets and small shops in Addis Ababa. The results obtained from this research could explain that canned foods under investigated were not microbiologically sterile.

According to warren *et al.*, (1998) the incidence of spoilage in canned foods is low, but when it occurs it must be investigated properly. Swollen cans often indicate a spoiled product. All canned foods in this study were not spoiled, however, they were not sterile, and also spoilage is not the only cause of abnormal cans. Some microorganisms that grow in canned foods do not produce gas and therefore cause no abnormal appearance of the can, nevertheless, they cause spoilage of the product (warren *et al.*, 1998).

These canned foods were maintained at ambient temperature at point of display. During warmer seasons in Addis Ababa spoilage may be aggravated. High summer temperature and low altitude increase the degree of spoilage (warren *et al.*, 1998)

Almost all canned foods contained heat resistant *Micrococcus* spp, *Bacillus* spp and some of them had *Staphylococcus* spp, Coliforms and yeasts. The presence of these flora indicated that these canned foods could be considered as under-processed (warren *et al.*, 1998). Mixed

microflora of viable bacterial rods and cocci usually indicate leakage that occurred sometime in the past (Warren *et al.*, 1998)

The pH values of all samples were >4.6, and could be considered as low acid canned foods. These pH values permit the growth of most pathogen. In addition, the products are nutritious and can support the growth of microorganisms. For these products, heat treatment after canning would be the only control factor with respect to microbial safety or spoilage. Factors such as pH and water activity of the heating medium greatly affect the survival of heated cells (Mafart, 2000). Moreover, if initial contamination is excessive, normal adequate processes are insufficient to eliminate contaminants. This could cause the consequent lengthening of the heat penetration time.

The aerobic mesophilic counts of canned fish products (Tuna and Sardine) were high for heat treated products. The mean microbial count of Tuna and Sardine was 4.14 (log cfu/g) and 4.22 (log cfu/g), respectively. Similar investigations carried out elsewhere showed that tins of sterilized canned fish were non sterile and cultures of seeded materials under specified conditions made it possible to establish the presence of residual microflora in the tins of fish (Todorov, 1977).

Fish is largely harvested from the marine origin and could be subjected to environmental contamination, including pathogens from the harvested site and on-board-ship handling practice (USFDA, 2001). The presence of coliforms and *Staphylococcus* in some samples of Sardines and Tuna indicated that these canned fish products were either under-processed or contaminated after heat-treatment. High protein content, and low acidic nature of the fish products (pH >5.6) could allow these contaminants to grow and produce toxins.

Since Sardine products were made of small fish, whose tail, head, and internal visceral organs were removed and Tuna products were made of minced fish flesh, contaminants could be protected by the high protein content and could receive less heat during canning process (Abdul *et al.*, 1999).

Both canned fish products were dominated by *Bacillus* spp and heat resistance *Micrococcus* spp. These *Bacillus* spp are common in marine environments (Elena *et al.*, 1999). Presence of *Micrococcus* spp might be due to contamination and under-processing.

Among the *Bacillus* isolates obtained from the fish products, *B firmus*, *B. cereus*, *B. licheniformis*, were the dominant. Studies showed that these species and other *Bacillus* species were often isolated from marine habitats (Boeye and Aerts, 1976). Another study also showed that strains of *B. subtilis*, *B. licheniformis* and *B. cereus* were common inhabitants of the Pacific Ocean habitats (Elena *et al.*, 1999). During canning process, spores of *Bacillus* spp could survive the heat treatment whereas non-spore formers and heat sensitive bacteria would be eliminated. When product formulation is incapable of inhibiting spore germination, they could spoil the canned products (IFT/FDA, 2003).

Borena Corned Beef and Beef-in-Jelly were locally produced canned meat products. Borena Corned Beef was a eured product, while Beef-in-Jelly was not. Both products were not commercially sterile. The mean pH value of the beef products was 5.90 and 5.70 and this was within the pH standards (5.5-6.0) as stated in Warren *et al.*, (1998).

The mean microbial load of canned Borena Corned Beef was 3.68 (log cfu/g). The flora of these canned products was dominated by *Bacillus* spp. Addition of sodium chloride and sodium nitrate in canned meat inhibits the outgrowth of bacterial spores. Lowering pH and increasing sodium chloride concentration enhance inhibitory action of sodium nitrate (Krumm *et at.*, 1998). Borena Corned Beef contained these additives for the same reason. Despite this fact, however, the product was not sterile. This might be due to improper curing. Cured canned meat products usually require less heat than uncured canned meat and, when spoilage occurs, it is usually the result of improper curing rather than inadequate heating (Krumm *et al.*, 1998). Even though sodium nitrate has inhibitory effect on spore growth, it can form carcinogens in meats. The amount of sodium nitrate added in to the canned product was not labeled. Additives that form carcinogens should be used at possible minimum amounts and their addition should follow the principle for extraneous additive chemicals, to be in a level as low as reasonably achievable (Nortermans *et al.*, 2003).

The microflora of canned meat products was dominated by *Bacillus* spp, *Micrococcus* spp and other gram positive rods. Coliforms were counted from one sample of Canned Beef-in-Jelly. Another study on canned meat showed that some canned meat products contained microbial load with a level of about 10^7cfu/g, mainly dominated by Enterobacteriaceae and *Staphylococcus* spp (Broek and Bijker, 1976). In the same study the contents of another can contained *Bacillus* spp at a level about 10^5/g. inadequate sterilization and errors in processing were suggested as possible causes (Broek and Bijker, 1976).

Bacillus spp were the dominant isolates in both canned meat products. The dominant strains were *B. licheniformis*, *B. cereus*, *B. macerans*, *B. subtilis* and *B. circulans*. In another study using the simplified identification scheme, 115 strains of *Bacillus* spp were isolated from spoiled canned foods. Most of the isolated strains belonged to the species *B. subtilis*, *B. megaterium* and *B. brevis* (Kotzekidou, 1996). Similarly *Bacillus* species were isolated from semi-canned meat, 50% of the bacilli studied were determined as *B. licheniformis*, 26% *B. subtilis*, 20% *B. pumilus* and 4% *B. cereus* (Petrova, 1975). These studies indicated that *Bacillus* species diversity was not uniform in canned foods.

Bacillus species can be the dominant spoilage flora in different foods such as traditional sauces (Mogessie Ashenafi, 1996). Due to their metabolic activity *B. pumilus*, *B. subtilis*, *B. cereus* (Feleke Moges and Mogessie Ashenafi, 2000), *B. licheniformis* (Montville, 1982) and *B. coagulans* (Fields *et al.*, 1977; Anderson, 1984) were identified as food spoilers.

Inadequate sterilization, errors in processing and addition of non-sterilized spices could be suggested as the possible explanation for the presence of these bacteria. Some of these strains of *Bacillus* spp were frequently isolated from some Ethiopian sauce spices that included *B circulans, B. licheniformis,* and *B. cereus.* Some of the isolates from the canned products were reported to show proteolytic activities (Feleke Moges and Mogessie Ashenafi, 2000). Other study also showed that from semi preserved canned sausages and their ingredients *B. subtilis, B. amyloliquefaciens* and *B. macerans* were predominantly isolated (Mitrica and Granum, 1997).

Asparagus, Mixed Vegetables and Farm Style Vegetables were canned vegetable products, which were investigated in this study. The two vegetable products (Mixed Vegetables and Farm Style Vegetables) contained varieties of vegetables as ingredients, but Asparagus canned product contained only Asparagus. Operation with poor sanitation in the packing environment could significantly increase the risk of contaminating raw materials of vegetables. Microorganisms may be found on vegetables and in the drains of the packing facilities and on the surface of sorting, grading and packing. Microbial cross contamination from other foods and non-food sources and contaminated surfaces may occur during loading, unloading, storage and transportation operation. Without good sanitary practices contamination could be excess. Excessive contamination of the product renders normal heating process insufficient and results in under-processing. This could be the possible explanation for the microbial load of these canned vegetable products.

The mean pH values of Asparagus, Mixed Vegetables and Farm Style Vegetables were within the standards for canned vegetables as given in Warren *et al.,* (1998). Special attention should be given to Mixed Vegetables because contents are consumed without further cooking according to the labeled instruction.

The canned soups considered in this study were condensed products and highly viscous. The presence of microorganisms in the soup products might be due to under-processing and viscosity nature of the product. Abdul *et al.,* (1999) using high viscous liquid (sodium-carboxy-methyl cellulose (CMC) as a model liquid food had showed that the slow heating zone (SHZ) in the process of heating migrated and eventually stayed in the region that is about 10.15% of the can height from the bottom. The slow heating liquid and the bacteria carried with it at these locations were exposed to much less thermal treatment than the rest of the product. Thus the microbial quality of these products could be related to this fact.

According to Warren *et al.,* (1998) the standard pH of vegetable and beef soups were 4.7-5.6 and Creamy Chicken Soup had 5.5-6.5 while the mean pH value of these soup products were 4.70 and 5.26, respectively.

Canned infant powder milk formulas contain many kinds of dry food additives to improve their nutritional values. They are added after heat-treatment (Richter and Vedamuthu 2001). Mild heat treatment, addition of non-sterilized additives and absence of good manufacturing practice could be possible reasons for the microbial load of these canned milk products.

The mean pH values of the milk products ranged between 6.17 and 6.41. The standard pH values for evaporated milk ranged from 5.9-6.3 (Warren *et al.*, 1998).

Bacillus spp, Micrococcus spp and other gram-positive rods dominated the microflora of canned powdered milk. The flora of *Bacillus* spp of the canned milk products was dominated by *B. sphaericus*, *B. cereus, B. pumilus* and *B. brevis*. A study in the assessment of the frequency and level of *Bacillus* spp contamination in the Sardinian diary products, the most frequently isolated species were *B. cereus, B. coagulans, B. subtilis* and *B. pumilus* (Cosentino *et al.*, 1997). These strains were isolated from human infections (Richter and Vedamuthu, 2001). If leftover milk consumed spores could germinate and infect the consumer.

Most isolates of *Bacillus* spp were reported as human infectious agents including *B. cereus*, *B. alvae, B, megaterium*, *B, coagulans*, *B. laterosporus*, *B. subtilis*, *B. sphaericus, B. circulans, B. brevis, B. licheniformis, B. macerans, B. pumilus, B. anthracis and B. thuringiensis* (Kenneth, 2005). Among them *Bacillus cereus* is the most prominent strains that cause food poisoning. Some group of *Bacillus* spp can produce heat stable toxins. Tavlor *et al.*, (2005) showed that majority of *Bacillus* spp such as *B. megaterium, B. simplex B. licheniformis, B. Cereus* and *B. firmus* were found to produce heat stable toxins. If the incoming raw materials in the canning process are contaminated with excess *Bacillus* spp the toxin production will be more and could reaches to lethal dose.

Antibiotic resistance is not a recent phenomenon. On the contrary this problem was recognized soon after the natural penicillin were introduced for disease control and the bacterial strains found to harbor antibiotic resistance genes. Antibiotics are products of the earth. More especially of soil they are by products of cellular metabolism of *Actinomycetes*, mainly *Streptomycetes* specics, produce tetracycline, aminoglycosides (streptomycin, and its relatives), macrolides (erythromycin

and its relatives), chloramphenicol, rifamycins, and most other clinically useful antibiotics that are not beta-lactam. But these resistant genes could be transferred to other soil bacteria (Harvey and Mason, 1998).

In some cases, the situation has now become alarming with the emergence of pathogenic strains that show multidrug resistance to a broad range of antibiotics. One of the most important examples of concern multiresistant strains is *S. aureus,* which is resistant to methicillin, cephalosporins, betalactams, macrolide, and aminoglycoside. Many of antibiotic resistance genes of *Staphylococci* are carried on plasmids that can be exchanged with *Bacillus spp* and *Streptococcus spp* and some are carried on transposons (Harvey and Mason, 1998).

Recently, much emphasis has been placed on bacterial gene transfer and acquisition of antibiotic resistance in the environment. Studies have investigated that genes present in bacteria in manure could transfer to indigenous soil bacteria. Resistant isolates belonging to the *Bacillus cereus* group from farm soil and manure were isolated, and screened for tetracycline resistance genes. All isolates carried transposons, which could transfer to other Gram positive bacteria (Agers *et al.*, 2002). Bradley *et al.,* (1998) also noted that transposons that encoded resistance to the antimicrobial drugs such as tetracycline were able to transfer between *B. subtilis, Enterococcus faecalis* and *B. thuringiensis.*

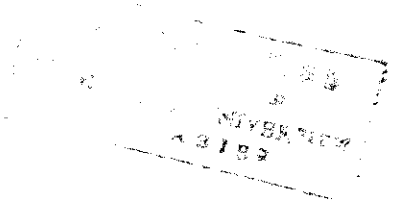
Bacteria are now more mobile than ever before. Bacteria in every environment where antibiotics are used are constantly evolving and exchanging genes that confer resistance to antibiotics. A bacterium that becomes resistant to antibiotics in a farm environment can easily find itself in canned foods through contamination and can later pass to the intestine of a person.

Among the total isolates of *B. cereus,* 32 showed multidrug resistance and the most frequent pattern was Aml/ Amp/ Cep/ Met/ PenG/ Sxt/ Tet. These resistance strains could harbor the gene from their natural environment and find themselves in canned foods through contamination. Almost all strains of *Bacillus cereus* in this study were susceptible to erythromycin, kanamycin chloramphenicol, streptomycin and vancomycin. In other studies from food poisoning incidents,

Bacillus cereus were totally susceptible to amoxicillin-clavulanic acid, gentamycin and a good proportion were susceptible to penicillin, vancomycin and tetracycline (Peter *et al.*, 2004).

Most isolates of *B. licheniformis* were resistant to amoxycillin, ampicillin, cephalothin, methicillin, penicillin G and tetracycline and all isolates showed multidrug resistance patterns for all of these antibiotics. However, almost all were sensitive to chloramphenicol, erythromycin, gentamycin, kanamycin, polymyxin B, streptomycin and vancomycin. The possible explanation for these resistance and multidrug resistance pattern of the different *Bacillus* isolates in canned foods could possibly be that they might have originally acquired the resistance gene from their natural environment.

According to Israel-Reches, (1983) naturally erythromycin resistance *Bacillus licheniformis* was found in 11 of the 18 isolates tested but was absent from a wide variety of other *Bacillus* strains. It was suggested that resistance might have arisen in the *Streptomyces* and spread to *B. licheniformis* and other gram-positive bacteria in the soil.



6. CONCLUSION AND RECOMMENDATIONS

Based on findings obtained in this study, the following conclusions and recommendations could be made.

The study of microbiological profile of some imported and local brands of canned foods should be further continued with multivariate parameters. This economic sector provides canned food products to the societies of different age groups and health conditions. Thus, this sector requires more attention and further additional studies including other canned foods items.

Aerobic spore-formers and anaerobic heat-resistance microorganisms have great role in poisoning and spoilage of various canned foods and cause subsequent outbreaks. Relating to these problems, the real effects of these microorganisms should also be evaluated along with other bacterial pathogens like *Listeria, Clostridium, Campylobacter, Yersinia, Vibrio* and *Pseudomonas species.*

The samples collected from Addis Ababa supermarkets and different shops provide information about some foods with microbial load that do not fulfill canned foods qualities. Thus, this study indicates that locally produced and imported canned food could be under-processed, excessive contamination raw materials or contaminated due to leakage.

Microflora of canned foods is heterogeneous. *Bacillus* spp, thermal resistant *Micrococcus* spp and other Gram positive rods were frequently encountered. Among them *Bacillus* spp and *Micrococcus* spp appeared to be the most dominant microflora. Since these canned foods are kept at room temperature in both market areas then products will spoil before their expiry dates.

Some cans did not show their date of manufacturing. This is against the interest of consumers, who should be informed about the status of food they buy. Therefore, authorized institutes should take the responsibility to check and take corrective measures.

Some canned foods are recommended to be consumed without heat treatment while others are not allowed to boil before consumption to simply keep the nutritional content and the original test and flavor of the product. The result of this study indicated that the microbial load of these canned foods could results in illness if they were consumed without heat treatment.

Ampicillin, amoxycillin and tetracycline are antibiotics, which are frequently resisted by most of *Bacillus* isolates. These drugs are mostly used for clinical purpose. This shows that resistance to these drugs is widely distributed in the environment. Considering the fact that resistance can be transferred to other pathogens, the use of these drugs could be ineffective soon.

These challenged canned foods could support the growth of pathogens. Thus, leftover canned foods must, kept and handled carefully to avoid contamination.

7. REFERENCES

Abdul, A. G., Farid, M. M., Chen, X. D. and Richards, P. (1999). An investigation of deactivation of bacteria in a canned liquid food during sterilization using computational fluid dynamics (CFD). *J. Food Eng.* **42**:207-214.

Agers, Y., Jenesen, L.B., Givskov, M. and Roberts, M. C. (2002). The identification of a tetracycline resistance gene tet (M), on a *Tn* 916-like transposons, in the *Bacillus cereus* group. *FEMS: Microbiol. Lett.* **214(2)**: 251-256.

Anderson, R. E. (1984). Growth and the corresponding elevation of tomato juice pH by *Bacillus coagulans J. Food Sci.* **49:** 647-649.

Aureli, P., Fenicia, L., Ferrini, A. M. (1984). Food poisoning caused by *Clostridium botulinum* type E. *J. hyg.* **63(6)**: 552-5.

Ball, C. O. (1923). *Thermal process time for canned food.* Bull. Natl. Res. Council 37(7), Natl. Res. Council Washington, DC.

Berkeley, C. W., Logan, N.A., Shute, L.A., Capey, A.G. (1984). Identification of *Bacillus* species. *Mthds. in microbiol.* **16:** 291-328.

Bennett, R. W. and Harman, S. M. (1988). *Bacillus cereus* food poisoning. **In:** *Laboratory diagnosis of infectious disease; principle and practice*, Pp. 83-93, (Balowa, A., Hauseler, W. J., Ohashi, Jr., M. and Turano, A. eds). Springer Verlag, New York.

Bigelow, W. D., Bohart, G. S., Richardson, A. C., Ball, C.O. (1920). *Heat penetration in processing canned foods.* Bull. No.16-L, Res. Labs. Natl. Canners Assoc., Washington DC.

Boeye, H. and Aerts, M. (1976). Numerical taxonomy of *Bacillus* isolates from North Sea sediments. *Int. J. Syst. Bacteriol.* **26:** 427- 441.

Bradley, J. H., Robert, E. A., Thomas, E. L. (1998). Conjugal transfer of *Tn* 916 in model soil systems. *Can. J. Microbiol.* **46(6)**: 542-549.

Broek, M. J. and Bijker, P.G. (1976). Food poisoning following consumption of canned meat prepared by a butcher. *Tijdschr Diergeneeskd* **101(7)**: 372-4.

Cosentine, S., Mulagia, A., Pisano, B., Palmas, F. (1997). Ineidence and biochemical characterization of *Bacillus* flora in Sardinian diary products. *Inf. J. Food Microbial.* **38(2-3)**: 235-8.

Dewanto, V., Wu, X., Adom, K. K., Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **50:** 3010–3014.

Dubell, C. (1960). Antony Van Leeuwenhoek and his "Little animals" Dover Publications. New York,

Efiuvwevwere, B. J and Atirike, O. L. (1998). Microbiological profile and potential hazards associated with imported and local brands of tomato paste in Nigeria. *J. Appl. Microbiol.* **84(3)**: 409-16.

Elena, P., Ivanova, M. V., Vysotskii, V. I., Olga, I. N., Natalia, M. G., Valery, V. M., Noboru, Y. S., Takahisa, T., Susumu, y. (1999). Characterization of *Bacillus* strains of marine origin. *J. Int. Microbiol.* **2:** 267-271.

Esty, J R. and Meyer, K. F. (1922). The heat resistance of spores of *Bacillus botulinus* and allied anaerobes. *J. Inf. Dis.* **31**:650-663.

Ethiraj, S. and Suresh, E. R. (1985). A note on the occurrence of *leuconostoc oenos* as a spoilage organism in canned mango juice. *J. food Microbiol.* **59(3)**: 239-42.

Feleke Moges and Mogessie Ashenafi (2000). Characterization of *Bacillus* spp from some spices and assessment of their spoilage potentials in various traditional Ethiopian sauces *SINET: Ethiop. J. Sci.* **23:** 87-101.

Fields, M. L., Zamora, A. F., Bradshet, M. (1977). Microbiological analysis of home canned tomatoes and green beans. *J. Food Sci.***42:** 931-934.

Gaze, J. E. (2005). Microbiological aspects of thermally processed foods. *J. Appl. Microbiol.* **98:** 1381–1386.

Gaze, J. E. (1992). *Food Pasteurization Treatments.* Gloucestershire, UK: Campden and Chorleywood Food Research Association, Technical manual no. 27.

Gregersen, G. (1978). Rapid method for distinction of Gram-positive from Gram-negative bacteria. *Euro. J. Appl. Microbiol.* **5:** 123-127.

Gonzalez, J. A., Gallardo, C. C., Gallego, A. R., Perez, M. J. and Rodriquez, L.A. (2002). Optimizing hot-fill processes (Sterilization process) in canned Chestnut Purce. *EJEAF Che.* **1(3)**: 1579-4377.

Harvey, J. and Mason, L. (1998). The use and misuse of antibiotics in agriculture. Soil Association, Bristol, UK.

Hartman, P. A. (1997). The evolution of food microbiology. **In**: *Food Microbiology*, Pp.413, (Doyle, M. P., Beuchat, L. R. and Monteville, T. J. eds), ASM Press, U.S.A.

Haves, A. J. (1983). The food and Drug Administration's role in the canned salmon recalls of 1982. *Appl. Environ. Microbiol.* **98(5)**: 412-5.

http:// www. simplot. com. au/ Hosting / corp /simsiteinsf /pages food% 20 Facts. Canning.

IFT/FDA (2003). Evaluation and definition of potentially hazardous foods. *Compr. Rev. Food Sci. and Food Safety.* **2:**8-14.

Israel-reches, M., Weinaruch, Y., Dubnau, D. (1983). Evolutionary relationship of the *Bacillus licheliformis* macrolide-lincosamide streptogramin resistance elements. *J. Molecul. Gen.* And *Geno.***149 (3)**: 362-367.

Jorgenson, J. H., Yurnidge, J. D., Washington, J. A. (1999). Dilution and disc diffusion method. **In**: *Manual and clinical Microbiology,* Pp. 1526-1543, (Murray, p. R., Baron, E. J., Pfaller, M. A., Tenover, F. C. and Yolken, R. H. eds), American Society for Microbiology, Washington, D. C.

Karmer, J. M. and Gilbert, R. J. (1989). *Bacillus cereus* and other *Bacillus* species. **In**: *Food Bacterial Pathogenesis*, (Doyle, M. P. ed.). Marcel Deker, Inc, New York.

Kenneth, T. (2005). *The Genus Bacillus.* University of Wisconsin-Medison Department of Bacteriology. Pp 519.

Kotzekidou, P. (1996). A microtiter tray procedure for a simplified identification of *Bacillus* spp. in spoiled canned foods. *J. Food Microbiol.* **13(1)**: 35-40.

Krumm, G. W., Lattuada, C. P., Johnston, R. W., Eye, J. G., Green, J. (1998). *Examination of heat processed, hermetically sealed (Canned) Meat and poultry products.* USDA/FSIS Microbiology Laboratory Guide book, (3rd ed.). Chap 10.

Levine, W.C, Bennett, R. W., Choi, Y., Henning, K. J., Rager, J. R., Hendricks, K.A., Hopkins, D.P., Gunn, R. A., Griffin, P. M. (1996). *Staphylococcus* food poisoning caused by imported canned mushrooms. *J. Infect Dis.* **175(5)**: 1263-7.

Mafart, P. (2000). Taking injuries of surviving bacteria in to account for optimizing heat treatments. *Int. J. Food Microbiol.* **55:**175-179.

Mallidis, C. G., Frantzeskakis, P., Balat, S. G., Katsa, B. C. (1990). Thermal treatment of aseptically processed tomato to paste. *Int. J. Food Sci. Tech.* **25:** 442-448.

Mitrica, L. and Granum, P. E. (1979). The amylase-producing microflora of semicanned food sausage; identification of the bacteria and characterization of their amylases. *Z Lebensm Unters. Forsch.* **169(1)**: 4-8.

Mogessie, A. (1996). Microbial spoilage of some traditional Ethiopian sauces at ambient temperatures. *Ethiop. J. Sci* **19:**207-16.

Montville, T. J. (1982). Metabolic effect of *Bacillus licheniformis* on *Clostridium botulinum*; implications for home canned tomatoes. *Appl. Environ. Microbiol.* **44(2)**: 334-8.

Nortermans, S., Barendsz, A. W., Rombouts, R. (2003). The evaluation of microbiological risk assessment in food production. **In**: *Microbiological risk assessment in food processing,* Pp. 36, (Brown, M. and Stringer, M. eds), Woodhead Publishing Limited Cambridge, England.

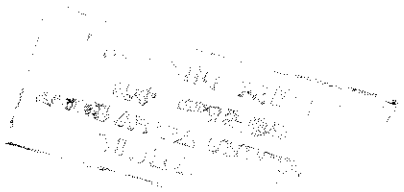
Odlaug, T. E. and Pflug, I. J. (1978). *Clostrdium butulinum* and acid foods. *J. food Prot.* **41:**566-573.

Palop, A., Raso, J., Pagan, R., Condon, S., Saler, F. J. (1999). Influence of pH on heat resistance of spores of *Bacillus coagulans* in buffer and homogenized foods. *Int. J. Food Microbiol.* **46:**243-249.

Peter, C. B. T., Nicky, M. S., Carlos, I. L., Marian, N. S., Felicia, N. S., Anatalio, E. R. and Leonard, F. P. Jr (2004). MICs of selected antibiotics for *Bacillus anthracis, Bacillus cereus, Bacillus thuringiensis* and *Bacillus mycoides* as determined by the Etest. *J. Clini. Microbiol.* **42(8)**: 3626-3634.

Petrova, L. (1975). Differentiation in culture of *Bacillus* species isolated from semi canned meat. *Vet. Med. Nauki.* **12(8)**: 82-88.

Ranken, M. D. (1997). *Food Industries Manual*, (24thed). Blackie Academic and Professional, London. Pp. 232.



Richter, R.L. and Vedamuthu, E. R. (2001). Milk and Milk products. **In**: *Compendium of methods for the microbiological examination of foods*. pp, 483 (Downes, F. P. and Ito, K., eds.), American public health Association (APHA), Washington, DC.

Robert, D., Hooper, W.Y., Greenwood, M. (1996). *Method for the examination of food for microorganisms for public health significance.* Practical Food Microbiology. (2nd ed). Public health laboratory service. London. Pp, 467.

Tavlor, J. M., Sutherland, A. D., Aidoo, K. E., Logan, N. A. (2005). Heat stable toxin production by strains of *Bacillus cereus, B. firmus, B. maganterium, B. simplex and B. licheniformis. FEMS. Microbial. Lett.* **242(2)**: 313-317

Todorov, I. (1977). Microbiological control of sterilized canned fish. *Vet. Med. Nauki.* **14(7)**: 55-61.

USFDA (2001). *Analysis of microbial hazards related to time/temperature control of foods for safety.* Food and drug administration center for food safety and applied nutrition. Department of health and human service.

USFDA (2000). *Kinetics of microbial inactivation for alternative food processing technologies.* Food and drug administration center for food safety and applied nutrition. Department of health and human service.

Warren, L. L., Albert, H. S. and Gayle A. L. (1998). *Examination of canned foods.* Bacteriological analytical manual. (8th ed). Pp. 234.

Wojciechowski, J., Szczepaniak, B., Morawiak, E., Piosik, L. (1976). Determination of relationship between heating value and the thermal resistance of microorganisms in canned meat. *Nahrung* **20(10)**: 883-887.