ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Microbiological Quality and Safety of Cream filled Cakes Produced in Various Pastries in Addis Ababa and the Antimicrobial Resistance of the Pathogenic Isolates

By
Geda Kebede

A Thesis Submitted to the School of Graduate Studies of the Addis Ababa University in partial fulfillment of the requirements for the degree of Master's of Science in Biology, Applied Microbiology

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TABLE OF CONTENTS

Pages

Contents

LIST OF TABLES

LIST OF FIGURES

ABBREVIATION

AAU-Addis ababa University

ACMSF- advisory committee on microbiological safety of foods

BSAC-British Society for Antimicrobial Chemotherapy

EB - Enterobacteriaceae

ECHCPD -European Commission Health & Consumer Protection Directorate

EHNRI-Ethiopian Health and Nutrition Research Institute

HACCP- Hazard Analysis Critical Control Point

ICMSF- International Commission on Microbiological Specifications for Foods

PCA-Plate count agar

SE- Staphylococcal enterotoxins

MDR-Multiple drug resistance

MSA- Mannitol salt agar

MSBB- Mannitol Selenite Broth Base

SBB - Selenite Broth Base

SCBB -Selenite cystin Broth Base

Spp. - species

TTB-Tetrathionate Base

TSB- tryptone soya broth

YGC- yeast extracts glucose chloramphenicol bromophenol blue

VRBA-Violet red bile agar

VRBGA-Violet red bile glucose agar

ABSTRACT

A total of 80 samples of cream cakes from ten different pastries in Addis Ababa were examined for their pH, moisture content, holding temperatures, bacteriological profile, amylolytic, proteolytic and lipolytic properties, presence of food-borne pathogens and their drug resistance pattern. The mean pH values of cream cakes from ten different pastry houses ranged from 5.43 to 6.64 and the mean moisture content ranged from 44.02% to 29.86 %. About 96.9 % of samples were stored within a temperature range of 15-25 ^{o}C. About 75.95% of the cream cakes had counts higher than log 8 cfu /g. Among bacterial strains isolated from different cream cake samples, *Bacillus* spp. (51.75%) dominated the microflora. Staphylococci and members of Enterobacteriaceae constituted 3.51% and 13.33 % respectively, of the microflora. About 45.85% of the isolates were amylolytic consisting mainly of *Bacillus* spp. About 1.85 % of them were proteolytic and none was lipolytic. This study indicated the need to train producers and handlers of cakes in basic principles of hygiene. Keeping cream cake at ambient temperatures must also be avoided.

Key words/phrases: *Cream cake, Microorganisms, Pastries, Quality, Safety.*

1. INTRODUCTION

Food-borne diseases caused by microorganisms are major public health problems. Food borne illnesses are prevalent in all parts of the world and human life suffering from it is enormous. For example, in the US, each year food-borne illnesses affect 6 to 80 million people, causing up to 325,000 hospitalizations and 9000 deaths, and cost about 5 billion US dollars (Altekruse *et al*., 1997; Buzby and Roberts, 1997).

Cases of food infection are most commonly encountered following picnics, festivals and banquets (Dolce, 1941). The foods most often responsible for illness are meat mixtures, ice cream, potatoes, salads and cream-filling for pies and cakes (Dolce, 1941).

Bakery products are important staple foods in most countries and cultures (Guynot *et al.*, 2003). They have been important part of a balanced diet for thousands of years. The production of bread and other bakery products has evolved from a primitive, cottage industry into a large-scale, modern manufacturing industry. From year to year, it is generating revenue of millions of dollars in different countries (Smith *et al.*, 2004).

Bakery products include pastry products which offer an excellent environment for the growth of bacteria owing to their favorable pH, water activity, nutrient availability and storage conditions (Uhitil *et al.,* 2004).

Many fillings of pastry products can support the growth of food pathogens, especially if they contain eggs or dairy products. Custard-filled products are a potential health hazard, due to the growth of *Bacillus cereus* and *Staphylococcus aureus* in them. The latter pathogen has also been implicated in food poisoning outbreaks from cream-filled bakery products (Smith *et al*., 2004). As indicated by Hardy (1942) this cream filling in pastry is an excellent culture medium for the growth of *S. aureus*.

Cream filled baked goods also have a notorious history as vehicles of food borne illness, particularly staphylococcal food poisoning. Staphylococcal food poisoning may be much more common than is recognized in different countries (Stewart *et al*. 2003). Therefore, foods posing outbreaks associated with bakery products have major public health significance (Bhatia and Zahoor, 2007).

The purpose of food safety is to prevent growth and toxin production from harmful microorganisms. Food safety is defined as protecting the food supply from microbial, chemical and physical hazards that may occur during all stages of food production, including growing, harvesting, processing, transporting, retailing, distributing, preparing, storing and consumption (WHO, 2007). The failure of applying food safety procedures during food production results in food borne illness (Stephens, 2007).

Food-borne outbreaks associated with cream-filled baked goods are attributed primarily to contamination by food handlers followed by inadequate refrigeration during manufacture and storage (Stewart *et al*., 2003). Many people are infected with enterotoxigenic staphylococci (Anunciacao, *et al*., 1995). As indicated in Stewart *et al*. (2003), 26-38.5% of food handlers are positive for the carriage of *S. aureus*

Microbial food poisoning such as staphylococcal food poising is common food-borne disease that occurs in most countries of the world (Anunciacao, *et al*.,1995).The vomiting and diarrhea that usually accompanies staphylococcal food poising lasts only a few hours to one day (Kerouanton *et al*., 2007). Most outbreaks occur after improper refrigeration of the cakes, probably due to lack of appreciation that this type of food is vulnerable to food poisoning microorganisms. Essentially all staphylococcal food poisoning outbreaks are a result of human contamination during the preparation of the food.

According to Ethiopian Health and Nutrition Research Institute /EHNRI (2003), the food processing industries, catering centers, hotels, supermarkets, food distributors, retailers, etc. have been established and operate without taking into account the ordinary basic hygienic conditions. The food safety control system in Ethiopia is little developed and is not able to support the production, supply and distribution of safe food to consumers. It is also unable to largely protect the public from possible sources of food borne diseases that could occur due to a failure to apply the well-known principles of food safety that have been established over many years, for instance, basic hygiene practices. Preliminary studies indicate that communicable diseases contribute 60-80% to the total possible causes of illness, among which food-borne diseases have been estimated to take the lion’s share (EHNRI, 2003).

Though there are large numbers of cream cake consumers in Ethiopia at different occasions in urban centers, the safety and quality of this product has not been assessed and published information is not available so far. Therefore, the purpose of this study was to evaluate the microbial load of cream cake as made available to the consumer in parts of Addis Ababa and determine the type and incidence of food-borne pathogen found therein.

OBJECTIVES

General Objective:

- To evaluate the microbiological quality and safety of cream cakes available to consumers in some pastries from Addis ababa.

Specific Objectives:

- To assess the microbial load of cream cakes.
- To determine the incidence of food-borne pathogens.
- To assess the amylolytic, proteolytic and lipolytic property of isolates
- To determine the drug resistance pattern of pathogens.

2. LITERATURE REVIEW

Millions of people fall ill and many do suffer from serious disorders, long-term complications or die as a result of eating unsafe food (WHO, 2001). Food-borne and water-borne diarrheal diseases are leading causes of illness and globally kill an estimated 2.1 million people annually, most of whom are children in developing countries (WHO, 2001).

Food can be contaminated with spoilage or pathogenic microorganisms. Spoilage organisms spoil the product so that it is not fit to eat or change the taste so that the product is not desirable. They are generally not harmful if ingested. Pathogenic organisms, however, can cause food infection or food poisoning. Food-borne diseases can arise either through the ingestion of food containing microorganisms, or by the ingestion of food containing toxins, which have been pre-formed by the microorganism (Needham, 2004).

According to ACMSF (2006), manufactured food products need to meet consumer drives such that they need to be of a desirable quality to consume, they should provide a positive contribution to a balanced diet of the consumer and, above all, they must be safe for consumption. One of the key targets is to achieve product safety with respect to the risks from food-borne pathogens

2.1. History of cakes

The history of cakes goes a long way back. Among the remains found in Swiss lake villages were crude cakes made from roughly crushed grains, moistened, compacted and cooked on a hot stone. Such cakes can be regarded as a form of unleavened bread, as the ancestor of all modern European baked products. Some modern survivors of these mixtures still go by the name 'cake', for instance oatcakes, although these are now considered to be more closely related to biscuits by virtue of their flat, thin shape and brittle texture. The most primitive peoples in the world began making cakes shortly after they discovered flour (http://www.foodmuseum.com/excake2.html).

Ancient Egypt was the first culture to show evidence of true skill in baking, making many kinds of bread. The Greeks had a form of cheese cake and the Romans developed early versions of fruit cakes with raisins, nuts and other fruits. These ended up in 14th century in Britain when immense cakes were made for special occasions. One was made with thirty kilograms of flour and contained butter, cream, eggs, spices and honey (http://www.foodmuseum.com/excake2.html). During the 19th century, technology made the cake-baker's life much easier. The chemical raising agent, bicarbonate of soda, introduced in the 1840's, replaced yeast, providing a greater leavening power with less effort (http://foodmuseum.com/excake2.html).

The popularity of home baking and the role of cakes in the diet have both changed during the 20th century; cakes remain almost ubiquitous in the western world. They have kept their image as luxury and maintained their ceremonial importance at weddings and birthdays (http://foodmuseum.com/excake2.html).

2.2. Types of cream filled pastry product

Cakes are chemically or mechanically leavened bakery products that are enjoyed by all ages. The ingredient in the formula affect cake volume, crumb grain evenness, tenderness, flavor, eating quality and shelf-life (Dogan *et al*., 2007).Filled bakery goods include Tarts such as fruit, jam, Pies such as meat, fruit; Sausage rolls, Pasties, Cakes such as cream, custard, Pizza and Quiche (Smith *et al*., 2004).

According to Abrahamson *et al*., (1952) as cited in Stewart *et al.,* (2003), cream filled baked goods can be categorized into three types on the basis of the basic procedures used for their manufacture. In the first category (for example, chocolate eclairs, Napoleons, and imitation cream pies), filling ingredients are combined, cooked, and dispensed into prebaked pastry, and then icing is added. The second category include custard pies where, pre-formed baked or unbaked pastry are filled with combined, uncooked filling, and then the entire pastry is cooked, cooled, and packaged. The third category (for example, Nesselrode pies), pre-baked pastry are filled with ingredients, some of which have been combined and cooked, but other ingredients are added without first being cooked, and there is no final baking of the completed pastry

The first category of cakes are readily exposed to recontamination of the bulk filling during cooling, transferring, and dispensing, whereas foods in the third category have the greatest likelihood of contamination, since some ingredients are not cooked at all. Although foods in the second category are essentially sterile after baking, post baking contamination on the top or on the surfaces of cut edges can lead to the growth of *S. aureus* and can result in food borne illness (Stewart *et al,*. 2003).

2.3. Component of cream cake pastry products

The compositions to prepare cream cakes are more or less similar. Some of the components of cream cakes support the growth of microorganisms. According to Castellani (1953), the cream filling components are water, starch, non-fat milk solids, salt, shortening, sucrose, whole egg and vanilla extract. Condensed milk, milk, egg yolk and corn starch were also stated in another study (Anunciacao *et al*., 1995).

2.4. Spoilage of pastry products

The spoilage problems of bakery products can be physical spoilage (moisture loss, staling), chemical spoilage (rancidity), and microbiological spoilage (yeast, mold, bacterial growth) (Smith *et al*., 2004). Many filled bakery products support the growth of food pathogens, especially if they contain egg or dairy products (Smith *et al*., 2004).

As indicated by Ooraikul (1991) in (Smith *et al*, 2004), microbiological spoilage is often the major factor limiting the shelf life of high and intermediate moisture bakery products. The most important factor influencing microbiological spoilage of bakery products is water activity (ACMSF,2006) but cakes rarely undergo bacterial spoilage due to high concentration of sugar which restricts availability of water (Jay, 2006).

Several factors influence the types and numbers of mold spores found on bakery products, including product type and relative humidity. The most common mold contaminants found on cake were *Wallemia sebi, Penicillium spp., Cladosporium spp*., *Aspergilli spp*, *P. notatum, P. expansum, and P. viridicatum* were the predominant spoilage molds (Seiler, 1976 cited in Smith *et al*., 2004).

2.5. Specific microorganisms of concern

2.5.1. *Salmonella* spp.

The salmonellae are small, Gram negative and non-spore forming rods. *Salmonella spp*. causing food-borne disease are commonly isolated from animals, polluted water; food contaminated by insect, animals feed and food products (Jay, 1996). *Salmonella spp*. may also be introduced into bakery products through a wide range of ingredients. Furthermore, *Salmonella spp*. can also be easily spread by cross-contamination when ingredients or finished bakery products are in contact with other animal foods or contaminated surfaces during production, storage, and transportation (Smith *et al*., 2004). Eggs are a major source of *Salmonella spp*. in bakery products (McCoy, 1975). *Salmonella spp*. have also been found in flour. Richter *et al*. (1993) reported that 1.3% of 4000 samples of wheat flour contained *Salmonella spp*. Since many raw bakery ingredients may contain *Salmonella spp*., constant vigilance is required to prevent their growth in these ingredients and to minimize cross-contamination of other ingredients and final baked products.

Most reported outbreaks of salmonellosis caused by eating contaminated bakery products are *S. enteritidis* PT4, *S. enteritidis* PT7, and *S. typhimurium*. In most outbreaks, eggs were confirmed as the suspected vehicle of transmission. The use of raw shell eggs in unbaked products has been the causative agent in several salmonellosis outbreaks. Outbreaks involving *Salmonella spp*. have been reported in cake mixes made with eggs (Smith *et al.*, 2004). As indicated by Costalunga and Tondo (2002), many kinds of foods were vehicle of *Salmonella spp*. Among the eight major sources of *Salmonella,* pastry products contributes to 17%.

Since raw eggs are common source of *Salmonella spp.*, the use of pasteurized egg is critical to control salmonellosis in bakery products (Smith *et al*., 2004). Processing conditions also play a role in the destruction of *Salmonella spp.*, strict temperature control is also critical in ensuring the safety of bakery ingredients containing fresh cream. The refrigeration (4^oC) of cream filled pastries has been shown to prevent the growth of *Salmonella spp.* (Smith *et al*., 2004). Therefore, temperature abuse at any stage during processing, distribution, and storage may compromise the safety of cream filled bakery products or their ingredients. The importance of strict temperature control cannot be overemphasized, as *Salmonella* spp. can grow as low as 6^oC. Additional barriers, such as potassium sorbate, may be used to limit the growth of *Salmonella spp.* in cream filled products (Wyatt and Guy, 1981). Strict personal hygiene, good manufacturing practices, ongoing education of personnel, and implementation of a HACCP approach are also critical to reduce cross contamination of products by *Salmonella* spp. and to limit the spread of this foodborne pathogen (Smith *et al*., 2004).

2.5.2. *Staphylococcus aureus*

Staphylococcus aureus, a Gram positive coccus, is the most frequently isolated pathogen among the society. *S. aureus* is ubiquitously widespread on the skin and mucosa of humans and animals as well as in the environment (Jay, 1996; Balaban and Rasooly, 2000). Humans harboring *S. aureus* are a major source of contamination of products during preparation or post preparation handling. The majority of staphylococcal food poisoning outbreaks result from the contamination of food during its preparation by the food handler. Humans are common carries of enterotoxigenic staphylococci in the nose and throat, or on the skin. In addition many infections can be caused by staphylococci, which is a more dangerous situation because they are present in large numbers and can easily be transferred to the food being handled. Many staphylococcal food poisoning outbreaks have resulted from foods prepared by food handlers with infections (Pereira *et al.,* 1994).

According to WHO's report two outbreaks of *Staphylococcus aureus* were associated with pastry products. One of these outbreaks probably originated from a food handler who may have been working while infected. In another outbreak, contributing factors included poor personal hygiene and inadequate temperature control (WHO, 2001).

In investigating the cause for outbreak of staphylococcal food poisoning involving 42 people who had eaten a meal at a restaurant in Brazil, swabs from nasal cavity, throat and under the fingernails of food handlers were cultured for the detection of enterotoxigenic staphylococci carriers. Four out of five of them were healthy carriers of enterotoxin A, B, C and D producing *Staphylococcus aureus*. These results indicated that the food handlers would have been the source of the food contamination (do Carmo *et al*., 2003).

Post preparation contamination is also possible from air, surfaces, and cross contamination, food contact surfaces, distribution and handling (Smith *et al*., 2004; Bhatia and Zahoor, 2007;Reij and Aantrekker ,2004). The bacterium can also grow in substrates with a low water activity of 0.86, over a wide temperature range of 7 to 47.8^{o}C, and at pH values ranging from 4 to 9.8 (Jay, 1996). Because of these properties, *S. aureus* can easily contaminate food and potentially grow in it. Therefore, analyzing cakes with perishable fillings for *Staphylococcus aureus* were mandatory because these bacteria are commonly used in the microbiological evaluation of foodstuffs as indicators of poor hygiene and poor food handling practices.

S. aureus is a major human pathogen that produces a wide array of toxins, thus causing various types of disease symptoms. Staphylococcal enterotoxins (SEs), are leading causes of gastroenteritis resulting from consumption of contaminated food (Balaban and Rasooly, 2000). In addition, SE is powerful superantigens that stimulate non-specific T-cell proliferation (Balaban and Rasooly, 2000).

Outbreaks of food poisoning, due to the eating of cream puffs, and other cream-filled bakery products are common. Most frequently the symptoms are due to a toxin produced by *Staphylococcus.* Although rarely fatal, food poisoning due to such toxins presents an important public health problem. The short incubation period, the acute onset, and the high incidence among those exposed produce public alarm to severity of the condition (Coughlin and Johnson, 1941).

Foods that are frequently implicated in staphylococcal food poisoning include meat and meat products, poultry and egg products, and salads such as egg, tuna, chicken, potato, macaroni and bakery products such as cream-filled pastries, cream pies, chocolate eclairs, sandwich fillings, and milk and dairy products (Bremer e*t al.*,2004).

Filled bakery products made from creams and custard fillings are seldomly implicated in outbreaks of *S. aureus* food poisoning in North America due to good manufacturing practices but they continue to be a major source of illness in many temperate countries where refrigeration is still a problem (Camo and Bergdall, 1990 as cited in Smith, *et al* 2004). Oh *et al.*, (2007) found the presence of *S. aureus* in 285 (8.6%) of the 3,332 samples collected. On the basis of the results obtained, the most frequently contaminated foods were cream-cakes, rice cakes with filling, and raw fish. Of these foods, the ones with the highest detection were cream-cakes (31.6%). It might be the origin of infection, because milk products, such as cream, are often implicated in staphylococcal food poisoning. These outbreaks showed that cream type fillings were excellent media for the growth of *S. aureus* (Smith *et al*., 2004 and Hardy, 1942).

The growth of *S. aureus* was found to be inhibited in fillings made from chocolate or cocoa (Cathcart and Merz, 1942 as cited in smith *et al*., 2004). D and L-serine have also been shown to inhibit the growth of this pathogen in cream fillings (Castellanni, 1953). Heating immediately after preparation can destroy *S. aureus* in fillings and filled products. Stricter *et al*.,(1936) as cited in smith *et al*.,(2004) reported that heating custard filled pastries previously inoculated with 10^5 cfu/g of *S. aureus* to 190.6^{o}C for 25 minutes resulted in complete inactivation. A strict temperature control (4^0C) of finished products and their ingredients is also critical to inhibit the growth and enterotoxin production of *S. aureus* (Bryan, 1976 in smith *et al*., 2003). Anunciacao, *et al*., (1995) reported that cakes held at room temperature resulted in food poisoning, while those held at cold did not. Cream filled cakes inoculated with 10^6 cfu/g of an enterotoxins producing strain of *S. aureus* did not support growth, nor was enterotoxin detected in cakes held under refrigeration. At room temperature, enterotoxin was detectable in cakes inoculated with 10^3 cfu/g in 18 hours. *S. aureus* can tolerate and even grow in products with high sugar content and a low a_w (Jay, 1996).Therefore, storage under strict temperature and humidity control is essential in preventing the growth of this pathogen (Anunciacao *et al*., 1995).

It is recommended that foods being prepared for groups should not be prepared by a food handler with any type of infection. The wearing of gloves during the preparation of the food would add insurance against contamination of the food, providing the food handler avoided touching any other part of the body with the hands during the preparation of the food (Pereira *et al.,* 1994).

3. MATERIAS AND METHODS

3.1. Sample collection

A total of 80 cream cake samples were purchased from ten different pastries in Addis Ababa, between November, 2007-June, 2008. All samples collected were immediately brought to the laboratory at Science Faculty, AAU for microbiological analysis. The holding temperatures of each sample were measured at the point of collection by inserting a sensing bulb of a laboratory thermometer until the temperature of the cream cake was stabilized.

3.2. Measurement of pH and Moisture content (%)

The pH value of each cream cake sample was determined by blending 10 g cream cake sample with 90 ml distilled water. The pH value of the homogenate was measured using a digital pH-meter. The moisture content of the cream cake was determined by allowing the samples to dry to constant weight at 35 ^{O}C.

3.3. Microbiological analysis

For microbiological analysis, 25 g of sample was aseptically removed and homogenized in 225 ml sterile 0.1 % (w/v) bacteriological peptone (Oxoid) in a flask for ten minutes. Serial ten-fold dilutions were also prepared by transferring one ml of the homogenized sample to 9 ml diluents.

3.3.1. Microbial enumeration

3.3.1.1. Aerobic mesophilic count (AMC)

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Plate Count Agar (PC) (Oxoid) plates. Colonies were counted after the culture media were incubated at 30-32^{O}C for 48 hours. Un-inoculated plates were also incubated at the same temperature to check for sterility of plating media.

3.3.1.2. Counts of Staphylococci

To carry out counting of Staphylococci the following was conducted: From appropriate dilutions, 0.1 m1 aliquots were spread-plated in duplicates on pre dried surfaces of Mannitol Salt Agar (Oxide) plates. The culture media were incubated at 30-32^{O}C for 36 hours after which yellow colonies were counted as staphylococci.

3.3.1.3. Counts of Enterobacteriaceae (EB)

To carry out counting of members of Enterobacteriaceae the following was conducted: From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Violet Red Bile Glucose Agar (Oxoid) plates. The culture plates were incubated at 30-32 °C for 20-24 hours after which pink to red purple colonies with or with out haloes of bile precipitation were enumerated as members of Enterobacteriaceae.

3.3.1.4. Counts of Coliforms

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Violet Red Bile Agar (Oxoid) plates. The culture plates were inculcated at 30-32°C for 24 hours after which purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms.

3.3.1.5. Counts of yeasts and Molds

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of yeast extract glucose chloramphenicol bromophenol blue agar (YGC) made from the following ingredients: yeast extract, 5g; dextrose, 20g; chloramphenicol, 0.lg; bromophenol blue, 0.0lg; agar, 15g; distilled water, 1000ml; pH, 6-6.4. The culture plates were incubated at 25-28°C for three to five days. Smooth (non-hairy) colonies without extension at periphery (margin) were counted as yeasts.

3.3.1.6. Bacterial spore count

To undergo spore count, after heating the sample for ten minutes in water bath of 80° C, 0.1 ml of appropriate dilutions were spread-plated in duplicates on the pre dried surface of PC (Oxoid) and colonies were counted after incubation at 30 to 32° C for 24 h.

The numbers of EB, AMC, yeasts, spore, coliforms and *Staphylococcus* from their respective duplicate countable plates were reported as log cfu/g calculated from the arithmetic mean of two replicates of sample preparation.

3.3.2 Flora analysis

After enumeration of aerobic mesophilic bacteria, about 10 to 15 colonies with distinct morphological differences such as colour, size and shape were randomly picked from countable plates and inoculated in to tubes containing about 5 ml Nutrient Broth (Oxoid). These were incubated at 30-32°C over-night. Cultures were purified by repeated plating and maintained on appropriate slants at 4°C and sub-cultured every four weeks until characterized using the following tests.

3.3.2.1. Cell morphology

From pure culture Gram staining was prepared on a microscope slide. The preparation was observed under light microscope using oil immersion objective. The morphological criteria considered during the observation were:

Cell shape: Rods, cocci

Cell arrangement: Singles, pairs, clusters, chains, and tetrads

Endospore: Present, absent

3.3.2.2. KOH Test

This test was done according to Gregerson (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 KOH solution for 10 seconds to 2 minutes and the inoculating loop was then raised slowly from the mass. When KOH solution became viscous, the thread of slime followed the loop for 0.5 to 2 cm or more. Typically, this was observed in Gram-negative bacteria. In cases of no slime the watery suspension did not follow the loop, the reaction was negative and this was seen in Gram-positive bacteria.

3.3.2.3. Oxidation Fermentation (O/F) test

The utilization of glucose by each isolate was assessed by O/F test as suggested by Hugh and Leifson (1953) to identify microorganisms that metabolize glucose fermentativly or oxidativly or that do not utilize glucose by either way.Ingredients (g/l): Peptone, 2g; yeast extract, 1 g; NaCl, 5g; K_2 HPO_4, 0.2 glucose,10g, bromothymol blue, 0.08g; agar, 2.5g;distilled water, 1000ml, pH, 7.10.

The freshly prepared medium (15ml amounts in 18 x 180 mm test tubes) was immediately cooled under tap water to avoid dissolution of oxygen in the medium. Broth culture of each isolate was inoculated into the medium by stabbing with a sterile straight wire to the bottom. Acid formation and growth regions were interpreted after 2 and 5 days of incubation at 30-32^{o}C

3.3.2.4. Catalase test

Catalase taste was carried out after young colonies were flooded with a 3% solution of hydrogen peroxide (H_2O_2). The formation of bubbles indicated the presence of catalase.

3.3.2.5. Cytochrome oxidase test

The young colonies on plate count Agar plates were flooded with 1% N, N, N, and N - tetramethyl-p-phenylenediammonium chloride in distilled water. The appearance of a blue color on the colonies within 30 seconds indicated a positive reaction. Any very weak or dubious reaction that occurred after 30 minutes was ignored.

3.4. Amylolytic, Proteolytic (Caseinase) and Lipolytic tests

3.4.1. Amylolytic test

For the determination of amylase producing microorganisms starch agar was used. The purified cultures from plate count agar were streaked on the pre dried surface of the plates. The streaked plates were incubated at 30^{o}C for two days. After incubation, starch hydrolysis was determined by the addition of Lugol's iodine solution. Organisms that form colonies surrounded by a clear zone were taken as amylase producers as exoenzyme.

3.4.2. Proteolytic (Caseinase) test

To determine if an organism can produce the exoenzyme caseinase, Nutrient Agar was supplemented with 2% of casein (Oxoid) (Azokpota, *et al.,* 2006). The purified cultures from plate count agar were streaked on the pre dried surface of the plates. The streaked plates were incubated at 30^{O}C for two days. Organisms that formed a zone of clearing around the bacterial growth were considered as producers of caseinase.

3.4.3. Lipolytic test

For the detection and enumeration of lipolytic microorganisms in cake samples, tributrin agar with the following ingredient was used: Peptone from meat,2.5g;Peptone from casein, 2.5g; Yeast extract,3.0g; Agar-agar12.0g, and glycerol tributyrate ,10 ml; Final pH 7.5 ± 0.1 (El-Diasty and Salem, 2007). The purified cultures from plate count agar were streaked on the pre dried surface of the plates. The streaked plates were incubated at 30 ^{O}C for three days. Lipolytic microorganisms produce colonies which are surrounded by clear zones on the medium which normally with opaque appearance.

3.5. Isolation and characterization of *Salmonella*

3.5.1. Primary enrichment

To test for the presence of *Salmonella,* 25g of cake sample was mixed with 225ml Buffered Peptone Water (BPW) and homogenized. From this homogenized sample 10 ml was transferred to 50 ml Tryptone Soya Broth (TSB) and incubated at 37^{O}C for 18-24 hours for the metabolic recovery and proliferation of cells which could have been injured during processing or to bring the number of target organisms to a detectable level.

3.5.2. Secondary enrichment

The following broths were employed for secondary enrichment: Selenite Broth Base(SBB) (Oxoid), Mannitol Selenite Broth Base (MSBB) (Oxoid) both suplmented with sodium biselenite ,Selenite cystin Broth Base (SCBB), Tetrathionate Base (TBB) (Oxoid) supplemented with iodide solution (20ml/l), The selective property of these broths lies in their ability to inhibit non-targeted microorganisms like Gram-positive bacteria and coliforms and permit the rapid multiplication of *Salmonella.* After pre-enrichment in Tryptone Soya Broth (TSB), l ml of culture was transferred into separate tubes each containing 10 ml of MSBB, TBB, SCBB and SBB. TBB was incubated at 43°C for 48 hours in water bath and the remaining were incubated at 37°C for 24 hours.

3.5.3. Solid media

MacConkey Agar (Oxoid), Salmonella-Shigella (SS) Agar (Oxoid) and Xylose Lysine Desoxycholate (XLD) medium (Oxoid) were used for plating purpose. A loopful of culture from each selective enrichment broth was streaked separately on to each of the solid medium and incubated at 37^{O}C for 18-24 hours. Based on their characteristic appearance, colonies suspected for *Salmonella* were picked from each selective medium and further purified and tested biochemically. Un-inoculated culture plates were incubated to check for sterility of the solid media.

3.5.4. Biochemical Identification

3.5.4.1. Triple Sugar Iron Agar (TSI) (Oxoid)

The butt was stabbed and the slant was streaked and incubated at $37^{O}C$ for 24 hrs to detect fermentation of glucose, sucrose and lactose as well as production of H_2S. The presence of alkaline (red) slant and acid (yellow) butt, with or without production of H_2S was considered as presumptive for *Salmonella.*

3.5.4.2. Lysine Iron Agar (LI) (Oxoid)

The butt was stabbed and the slant was streaked and incubated at $37^{o}C$ for 24 hours. Salmonella produces the enzyme lysine decrboxylase that produces an alkaline reaction (purple color) throughout the medium. Due to the production of H_2S, an intense blackening of the medium will be seen that is a positive reaction for *Salmonella.*

3.5.4.3. Urea Agar (Oxoid)

The slant was streaked and the tube was incubated at $37^{O}C$ for 24 to assess the hydrolysis of urea. No color change in the slant was considered as negative and thus presumptive for *Salmonella*.

3.5.4.4. Simmons Citrate Agar (Oxoid)

The slant was streaked and the tube was incubated at $37^{O}C$ for 24 hrs to determine citrate utilization as a sole source of carbon. The presence of growth and color change from green to blue was considered as presumptive for *Salmonella.*

3.5.4.6. Mannitol broth

Ingredients: Mannitol,10gm; peptone,10gm; NaCl, 5 gm;phenol red,0.024gm; distilled water,1000ml and the pH was adjusted to 7.2. A colony was picked and inoculated into the broth and incubated at 37 ^{O}C for 18-24 hours to test fermentation and production of gas. Fermentation tubes containing inverted Durham's tubes were used to detect gas production.

3.5.4.7. Sucrose broth

Ingredients: Sucrose, 10gm; peptone,10gm; NaCl, 5gm; phenol red, 0.024gm; distilled water,1000ml and the pH was adjusted to 7.2. A colony was picked and inoculated into the broth and incubated at 37 ^{O}C for 18-24 hours to test fermentation and production of gas. Fermentation tubes containing inverted Durham's tubes were used to detect gas production.

3.5.4.8. Glucose broth

Ingredients: Glucose, 10gm; peptone, 10gm; NaCl, 5gm; phenol red,0.024gm; distilled water,1000ml and the pH was adjusted to 7.2. A colony was picked and inoculated in to the broth and incubated at 37^{O}C for 18-24 hours to test fermentation and production of gas. Fermentation tubes containing inverted Durham's tubes were used to detect gas production.

To ascertain sterility of the media for biochemical tests, all tubes with media were pre-incubated at 37°C for 18-24 hours.

3.6. Antimicrobial sensitivity testing for *Salmonella* species

Antimicrobial susceptibility testing was done for *Salmonella* isolates. The slanted cultures were sub cultured and purified. The pure colonies were inoculated into Nutrient Broth and incubated at 32^{O} C for 18-24 hours. After incubation, the turbidity of the culture was adjusted to 0.5 McFarland Standard to bring the cell density to approximately 10^7-10^8cfu/ml. The 0.5 McFarland turbidity standard was prepared by mixing 0.05ml $BaCl_2$ (1%) with 9.95 ml $H_2 SO_4$ (1%) (Andrew, 2001).

Muller-Hinton (MH) (Oxoid) plates were prepared and warmed to ambient temperature for plating. A sterile cotton swab was dipped into the standardized suspension. The culture was spread evenly over the entire surface of the Muller-Hinton agar plates by swabbing in three directions at 90^0 of each spreading. The plates were allowed to dry before applying antimicrobial disks.

The following standardard Oxoid drug discs were used: Ampicillin (Amp), (10iu); Chloramphenicol (Chl), (30µg); Gentamycin (Gen), (10µg); Tetracycline (Tet) , (30µg); Ciproflaxin (Cip), (5µg); Ceftriaxone (Cef), (30µg); Penicillin G (Pen), (10ug); Streptomycin (Str), (10µg); Kanamycin (Kan),((30µg); Polymaxin B (Pol),(100iu); Amoxicillin, (Amo), (2 µg). For the purpose of interpretation, those intermediate cases were considered sensitive.

3.7. Statistical analysis

To see if there was significant variation in counts within samples of each pastries, sample coefficient of variation (CV) was calculated. Values greater than 10% were considered as significant.

4. RESULT

4.1. pH and Moisture content (%) of Cream cake

The mean pH values of cream cakes from ten different pastry houses were between 5.43 and 6.64 with some difference when observed for each pastry house. Variations within samples of cream cake at each pastry house were significant (CV>10 %) (Table 1). Variations regarding pH within all cream cakes from all pastry house were also significant (>10%) (Data not shown).

Table 1. The pH and moisture (%) values of cream cake

Sample source	pH					Moisture content (%)				
	Min	Max	Mean	S.D.	% CV	Min	Max	Mean	S.D.	% CV
A	3.92	7.22	5.69	1.10	19.33	25.02	38.16	31.22	4.18	13.39
B	3.55	7.57	6.35	1.44	22.68	27.40	52.80	35.86	7.75	21.61
C	3.63	7.46	5.92	1.45	24.49	28.84	51.24	35.36	7.65	21.63
D	3.69	7.06	5.76	1.38	23.96	27.00	55.20	44.02	11.75	26.69
E	3.80	7.11	6.24	1.07	17.15	25.04	61.60	37.36	12.85	34.40
F	3.22	7.76	6.64	1.53	23.04	18.24	47.85	34.74	9.24	26.60
G	3.24	7.40	5.90	1.40	23.73	19.40	59.64	35.99	11.68	32.45
H	3.23	7.61	6.30	1.49	23.65	19.28	56.32	39.18	11.04	28.18
I	3.21	7.11	6.23	1.21	19.42	22.24	43.52	33.27	8.98	26.99
J	3.01	6.69	5.43	1.26	23.20	16.32	44.72	29.86	9.24	30.94

The mean moisture content of cream cakes from each pastry house was in the range of 29.86 % to 44.02 % with some difference when observed for each pastry house. Variation within each pastry house regarding the water content was significant (CV>10)(Table 1). In addition, variation within all sampled cream cake from all pastry houses were also significant (CV>10) (Data not shown).

4.2. Holding temperature of cream cakes

The various cream cakes were held at different temperatures when they were made available for consumption. About 45 % of the samples were within the temperature range of 15-20 ^{O}C and about 52 % were within the temperature range of 21-25 ^{o}C. The remaining 1.67 and 1.43 % were below 15 and above 25 ^{O}C, respectively. In general, 97 % of these samples were stored within a temperature range of 15-25^{O}C (Table 2).

Table 2. Holding temperature (^{O}C) of cream cakes

Sample source	*Temperature range (%)*			
	< 15	15-20	21-25	> 25
A		83.33	16.67	
B		50.00	50.00	
C		33.33	66.67	
D		42.86	57.14	
E	16.67	33.33	50.00	
F		33.33	66.67	
G		57.14	42.86	
H		28.57	71.43	
I		42.86	42.86	14.28
J		42.86	57.14	
Mean	1.67	44.76	52.14	1.43

4.3. Microbiological Spectrum of Cream cakes

A total of 570 bacterial isolates were isolated from cream cakes and characterized to various genera and bacterial groups. Different genera constituted the dominant microflora (Table 3). Gram-positive organisms dominated (60.88%) the microflora. The majority were *Bacillus* spp. (51.75%) followed by other Gm +ve rods (4.21%) and *Micrococcus* (3.51%) and *Staphylococcus* spp. were encountered at low levels (1.40%). *Aeromonas* (19.82%) while members of the *Enterobacteriaceae* (13.35%) were also encountered frequently (Table 3).

Table 3. Frequency distribution (%) of dominant bacteria in cream cakes collected from different pastries from Addis ababa, from November, 2007-June, 2008.

Sample source	No. of isolates	Bacillus Spp.	Aeromons	Enterobac teriacae	Acineto-bacter	Micro-coccus	Staphylo-coccus	Pseud monas	Other Gm +ve rods
A	53	39.62	26.42	20.75	1.89	-	-	3.77	7.55
B	55	43.64	20.00	27.27	3.64	-	1.81	-	3.64
C	54	37.04	24.07	9.26	-	12.96	1.85	3.70	11.12
D	67	53.73	26.87	10.45	1.49	1.49	-	4.48	1.49
E	50	60.00	14.00	10.00	8.00	-	-	-	8.00
F	52	55.77	7.69	21.16	5.77	7.69	-	-	1.92
G	60	46.67	30.00	8.33	6.67	3.33	-	-	5.00
H	67	68.66	16.42	1.49	1.49	5.97	4.48	-	1.49
I	54	70.37	3.70	12.97	5.56	1.85	1.85	1.85	1.85
J	58	39.66	25.86	15.52	12.07	1.72	3.45		1.72
Total	570	51.75	19.82	13.35	4.56	3.51	1.4	1.4	4.21

Table 4. Frequency distribution (%) of Gram positive and Gram negative dominant bacteria in cream cakes collected from different pastries from Addis ababa, from November, 2007-June, 2008.

Gram Positive isolates	%	Gram negative isolates	
Bacillus Spp	51.75	Aeromonas	19.82
Micrococcus	3.51	Enterobacteriaceae	13.55
Staphylococcus	1.40	Acinetobacter	4.56
Other gram +ve rods	4.21	Pseudomonas	1.40
Total	60.88		39.32

The aerobic mesophilic count of cream cakes from all pastries ranged from log 7 to log 10 cfu/g and about 55.7 % of the sample had counts ≥ log 8 cfu/g, 18.99 % had counts ≥ log 9 cfu/g and 1.26 % ≥ log 10 cfu/g (Fig.1).

Enterobacteriaceae and coliforms were encountered at a level between log 5 to log 9 cfu/g. About 73% and 88% of Enterobacteriaceae and coliforms respectively were found from log 6 to log 7 cfu/g. About 69% staphylococci were encountered in range of log 4 to log 5 cfu/g. Even though, yeast and molds were found in the range of log 3 to log 7, about 79 % were found in the range of log 4 to log 5 cfu/g. About 80% of spores were found in range of log 2 cfu/g to log 3 cfu/g. Regarding the variation in counts obtained from all cream cakes, significant variation was not observed in aerobic mesophilic counts (CV<10) while small variation was observed in Enenterobacteriaceae and coliform counts in all cream cakes(CV>10). Significant variations were noted in counts of staphylococci, yeast and molds and spores from all cream cakes (CV=14.83-23.01%) (Table 4).

Table 5. Microbial counts (log cfu/g) of cream cakes sampled from some pastries of Addis ababa, from November, 2007-June, 2008.

Bacterial groups	*Min*	*Max*	*Mean*	*SD*	*%CV*
Aerobic mesophilic count	*7.16*	*10.37*	*8.53*	*0.66*	*7.76*
Enterobacteriaceae	*5.60*	*9.41*	*7.46*	*0.80*	*10.76*
Coliforms	*5.00*	*9.24*	*7.07*	*0.77*	*10.89*
Staphylococci	*3.19*	*7.63*	*5.29*	*0.94*	*17.69*
Yeast and Mold count	*3.40*	*7.05*	*5.24*	*0.78*	*14.83*
Spore count	*2.00*	*6.22*	*3.35*	*0.77*	*23.01*

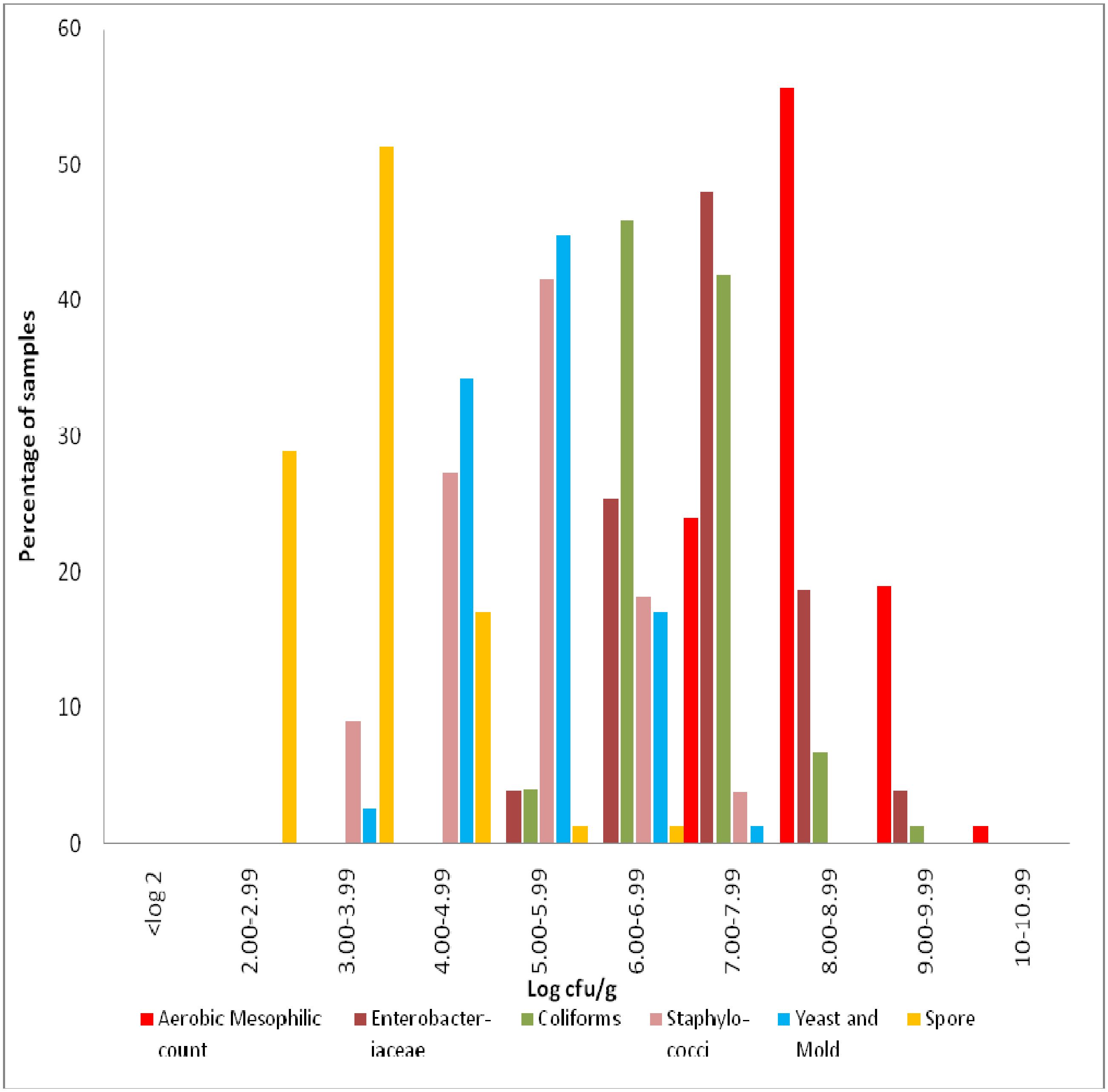


Fig.1. Distribution of microbial counts (log cfu/g) in cream cake samples, from November, 2007-June, 2008.

Table 6. Microbial counts (log cfu/g) of cream cakes from some pastries (A-E) of Addis ababa, from November, 2007-June, 2008.

Bacterial groups	*A*					*B*					*C*					*D*					*E*				
	Min	Max	Mean	SD	%CV	Min	Max	Mean	SD	%CV	Min	Max	Mean	SD	%CV	Min	Max	Mean	SD	%CV	Min	Max	Mean	SD	%CV
Aerobic mesophilic count	8.24	10.37	9.02	0.74	8.20	7.18	9.43	8.58	0.79	9.20	7.63	9.33	8.64	0.50	5.79	7.75	9.27	8.37	0.50	5.97	7.31	9.08	8.48	0.55	6.49
Enterobacteriaceae	6.93	9.34	6.93	0.74	10.68	5.88	7.93	7.30	0.79	10.84	6.98	8.98	7.87	0.74	9.40	5.63	7.99	6.98	0.78	11.17	6.40	8.33	7.31	0.62	8.48
Coliforms	6.54	8.37	7.42	0.73	9.84	5.90	7.88	6.74	0.71	10.46	6.18	8.86	7.41	1.01	13.63	6.28	7.91	6.71	0.56	8.35	6.00	8.23	7.01	0.82	11.70
Staphylococci	3.24	7.23	5.38	1.32	24.54	3.89	7.09	5.49	0.98	17.93	3.65	7.63	5.57	1.21	21.72	3.19	5.33	4.43	0.84	18.96	3.63	6.80	5.34	1.05	19.66
Yeast and Mold count	4.70	6.49	5.53	0.52	9.40	4.18	6.14	5.03	0.82	16.32	4.00	5.64	4.96	0.70	14.11	4.44	6.44	5.48	0.77	14.05	4.65	6.06	5.19	0.49	9.44
Spore count	2.18	4.30	2.92	0.66	22.60	2.30	4.54	3.14	0.76	24.68	2.65	3.93	3.25	0.46	14.15	2.74	4.40	3.28	0.52	15.85	2.48	6.22	3.66	1.12	30.60

SD-standard deviation, %CV-Percent coefficient of variation

Table 7. Microbial counts (log cfu/g) of cream cakes from some pastries (F-J) of Addis ababa, from November, 2007-June, 2008.

Bacterial groups	F					G					H					I					J				
	Min	Max	Mean	SD	%CV	Min	Max	Mean	SD	%CV	Min	Max	Mean	SD	%CV	Min	Max	Mean	SD	%CV	Min	Max	Mean	SD	%CV
Aerobic mesophilic count	7.96	9.06	8.52	0.42	4.93	7.30	10.14	8.57	0.98	11.44	7.20	9.13	8.33	0.58	6.96	7.68	9.61	8.51	0.66	7.76	7.16	9.27	8.27	0.76	9.19
Enterobacteriaceae	6.53	9.31	7.75	0.88	11.35	6.30	8.11	7.34	0.67	9.13	6.93	8.17	7.40	0.48	6.47	5.60	8.05	7.11	0.89	12.52	6.27	9.41	7.45	1.15	15.44
Coliforms	6.40	9.24	7.36	0.91	12.36	6.41	7.93	7.03	0.49	6.97	6.30	7.91	7.08	0.49	6.92	5.00	7.66	6.75	0.85	12.59	6.22	8.56	7.08	0.92	12.99
Staphylococci	3.70	6.61	5.58	0.99	17.74	4.18	6.56	5.28	0.73	13.83	4.40	5.78	5.03	0.54	10.74	4.10	6.57	5.24	0.78	14.89	4.20	6.29	5.47	0.70	12.80
Yeast and Mold count	4.48	6.60	7.24	0.74	10.22	4.00	6.51	4.92	0.86	17.48	4.18	6.53	5.40	0.91	16.85	3.40	6.55	5.10	0.99	19.41	3.60	7.06	5.51	0.98	17.79
Spore count	2.00	4.30	3.44	0.75	21.80	2.60	4.45	3.61	0.67	18.56	2.18	4.00	3.12	0.70	22.44	3.08	5.70	3.85	0.90	23.38	2.00	4.65	3.19	0.84	26.33

SD-standard deviation, %CV-Percent coefficient of variation

4.4. Amylolytic, Proteolytic and Lipolytic Microbial isolates from cream cake

Among the total microbial isolates, 45.85 % were amylolytic, 1.85 % were proteolytic and none was lipolytic. Out of the amylolytic isolates, about 38 % were *Bacillus* spp.

Table 8. The percentage (%) of Amylolytic, Proteolytic and Lipolytic bacterial isolates from cream cake

Sample source	No .of isolates	%		
		Amylolytic	Proteolytic	Lipolytic
A	53	43.40	-	-
B	55	47.27	-	-
C	54	35.19	1.85	-
D	67	52.24	-	-
E	50	56.00	-	-
F	52	44.23	-	-
G	60	43.33	-	-
H	67	40.30	-	-
I	54	50.00	-	-
J	58	46.55	-	-

4.5. Drug resistance of *Salmonella* spp.

From a total of 80 cream cakes examined, 5 samples were positive for *Salmonella*. The 10 isolates of *Salmonella* obtained from different combinations of enrichment and selective media were tested for an array of eleven different antimicrobials. Most of the isolates (nine) are resistant to Penicillin G, none was resistant to Tetracycline, Streptomycin, Ciproflaxin, Ceftriaxone and Polymaxin B. Seven isolates showed MDR.

The isolates showed resistance to three and four different antimicrobials (Table 8). The resistance pattern, Pen/Amp/Amo, was dominant in those isolates resistant to three antibiotics.

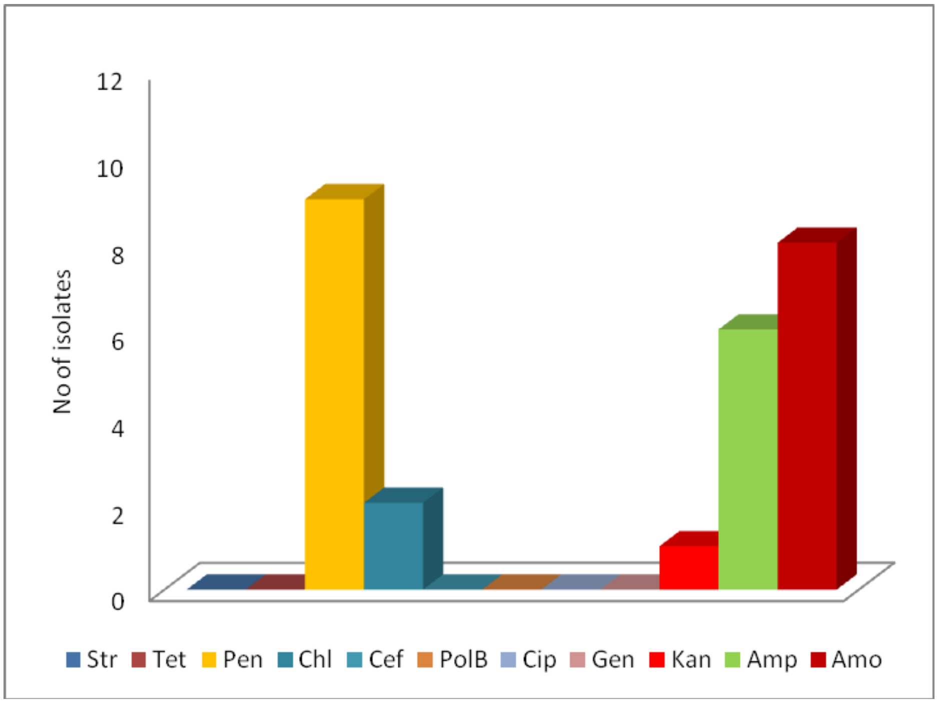


Fig 2. Resistance/susceptibility of *Salmonella* isolated from cream cakes' to antibiotics

Table 9. MDR pattern in *Salmonella* isolated from cream cake

No. antibiotic resisted	*No. resistant isolates*	*The frequent pattern of resistance*	
		No. of isolates	*Resistance pattern*
Three	5	4	Pen/Amp/Amo
		1	Pen/Chl/Amo
Four	2	1	Pen/Chl/Amo/Amp
		1	Pen/Kan/Amp/Amo

5. DISCUSSION

Microbiological quality and safety of cream cakes available to consumers in some pastries from Addis Ababa was evaluated in this study. The majority of the investigated food items in this study yielded high microbial load. The food handling, holding (temperature abuse) and serving operations, revealed inadequacies concerning hygiene and may have contributed to the high microbial load.

The mean pH value of all samples was 6.4 with a range of 5.43 and 6.64, hence cream cakes may be categorized under non acidic bakery product (Smith *et al*., 2004).These pH range may make these products susceptible to bacteria, mold and yeast growth (Jay,1996). In addition, as far as a cake has cream as component of product, this milk product-based cake is a good nutritional environment for microbial growth due to high nutrient value. Smith *et al*., (2003) also indicated that any bakery products and their ingredients having a pH of >4.6 would create a condition that is conducive to the growth of pathogenic bacteria. For example, the pH of custard used in many filled baked products is 5.8–6.6 and is an ideal for the growth of *Salmonella* spp.

The moisture content values in this study were in the range of 29.86 % to 44.02 %. According to Smith *et al*., (2003), microbiological spoilage is the main concern of intermediate and high moisture products. High moisture unfilled and filled bakery products have been implicated in outbreaks of foodborne illness. Hence, most pathogenic microorganisms require higher $a_{w,}$ the high moisture content of cream cakes might make them susceptible to microbial spoilage. Jay (1996) also mentioned that the percentage water content of cake is 19.3 which is lower than what have recorded in cream cake. However, as cakes may contain a considerable amount of simple sugars as sweeteners, most of the moisture would be bound by them resulting in a lower a_w.

Most bakery products, with the exception of cream, custard, and meat filled products, are held at ambient temperature. However, such storage conditions may be conducive for microbiological growth and may compromise safety (Smith *et al*., 2003). Where as in this case about 97 % of the pastry product were stored at ambient temperature varying from 15-25 ^{O}C. Therefore, since the samples contained cream, holding them at this temperature could create conducive environment for microbial growth. Though, products, such as cream, meat, and cheese filled cakes have an established history as vehicles of foodborne illness, holding at refrigeration temperatures will delay microbiological growth in these filled products but it may not be sufficient to prevent the growth of psychrotrophic pathogens, such as *Listeria monocytogenes* (Smith *et al*., 2003). Thus, this particular temperature range could promote rapid growth of common microorganisms and many pathogens with prolonged holding periods, whereas pathogenic bacteria have limited opportunity to proliferate in large numbers if the holding time is short.

About 76 % of samples in this study had aerobic mesophilic counts of ≥ log 8 cfu/g. But, according to ICMSF (2001), aerobic mesophilic counts ≥ 10^7 cfu/g are considered to be of unsatisfactory quality. Thus, the microbial load all samples were beyond the acceptable microbiological limits and this is indicative of poor hygiene or food handling practices. Storage at ambient temperature for long period of time may be the reason for high aerobic mesophilic count.

In this study, about 73 % of the samples had count of Enterobacteriaceae from log 6 to log 7 cfu/g. The count of coliforms was in between log 5 to log 9 cfu/g. About 88% were found to contain coliforms at a range from log 6 to log 7 cfu/g. According to ICMSF (2001), Enterobacteriaceae and coliforms are useful indicators of level of hygiene and of post-processing contamination of heat processed foods. The presence Enterobacteriaceae in high numbers $\geq 10^4$ cfu/g in ready-to-eat foods indicates that an unsatisfactory level of contamination has occurred or there has been under processing (inadequate cooking).Low numbers of coliforms are also permitted to the number of 100 cfu/g (Jay, 2006). Long holding time at ambient temperature, may help Enterobacteriaceae to dominate the flora

The count within samples about 69 % Staphylococci were found at log 4 to log 5 cfu/g. Contamination of ready-to-eat foods with staphylococci is largely a result of unhygienic handling. Unsatisfactory levels of staphylococci indicate that time/temperature abuse of a food is likely to have occurred following improper handling during food preparation. According to ICMSF (2001) counts $\geq 10^4$ cfu/g are considered as potentially hazardous as foods with this level of contamination may result in food-borne illness if consumed. Stapylococcal enterotoxins are quite heat resistant, not easily inactivated during food processing, storage, distribution or preparation. Therefore, if enterotoxinogenic Staphylococci are able to grow in products to high numbers ($>10^5$ to 10^6 cfu/g) before they are killed, there is still a risk of intoxication from consumption of the product (ECHCPD, 2003).

The values of yeast count in this study were in the ranges from log 3 cfu/g to log 7 cfu/g. Of this about 79 % were found in the range of log 4 to log 5 cfu/g. Though there are yeasts that can grow at low a_w, such as osmophilic yeasts, generally, yeast and molds are problems mainly in intermediate and high moisture bakery products such as cream cakes (smith *et al*, 2003). Therefore, the presence of such count of yeast in cream cake may shorten the shelf life of the product due to Proteolytic and lipolytic property.

Out of the collected cake samples about 80 % of them contained spores in the range of log 2 cfu/g to log 3 cfu/g. Since spores germinate into vegetative cell in favorable environmental conditions it may be one source for spoilage of cream cake. *Bacillus* spp. dominate the microflora of cream cake (51.75%); it was consistently isolated in considerable numbers from all samples. This might be due to the widespread distribution of the genus in nature with the high probability of contaminating any food (Sorokulova, *et al*., 2003). The source of this *Bacillus* spp. may be soil, dust and vegetation. Foods associated with *Bacillus* spp. include cereal products, spices, raw or dried processed foods, milk and dairy products, and bakery products (ICMSF, 2001).

The spores of *Bacillus* are resistant to heat and some can survive the baking process, because the temperature in the centre of the bakery product remains at a maximum of 97–100^{o}C for only a few minutes (Farmiloe *et al*. 1954 as cited in Sorokulova, *et al*., 2003). Spores that have survived baking when exposed to a warm, moist environment can germinate and cause rope spoilage of bread. According to ICMSF (2001), counts of pathogenic *Bacillus* spp. $\geq 10^4$ cfu/g are considered potentially hazardous as consumption of foods with this level of contamination may result in food-borne illness. Some strains of *B. cereus, B. subtilis and B. licheniformis* have been reported to be causal agents of food poisoning (Gilbert *et al*. 1981 cited in Sorokulova, *et al*., 2003).

Though, the high dominance of *Staphylococcus* is expected in cream cakes due to the holding temperatures and inadequate handling, Staphylococci were not as dominant as *Bacillus*. This may be due to less competitive nature of Staphylococci in the presence of high and complex background microbial flora. According to Bremer *et al.,* (2004) *Staphylococcus* is considered to be poor competitor in complex microbial populations and is frequently inhibited or overgrown by other faster growing microorganisms (spoilage organisms) in foods. Therefore, foods that present the greatest risk of Staphylococcal food poisoning are those where the normal microflora has previously been destroyed (such as cooked products) or inhibited (such as foods that contain a high concentration of salt) but that have subsequently been contaminated by *S. aureus.*

From the sample collected in this study and tested for their amylolytic activity, 45.85 % contained amylolytic isolates dominated by *Bacillus* spp. In addition, among the tested isolates for lipolysis, none were lipase producers. Although the hydrolytic property of our molds and yeasts was not determined in this study, because they did not constitute the dominant flora, their relative high count may contribute to the proteolytic and lipolytic spoilage of cream cakes. Molds and yeasts were the main spoilers in cheese products due to their proteolytic and lipolytic activities by breaking down their components and liberating different acids and gas with subsequent change of their odor and flavor (El-Diasty and Salem, 2007).

Salmonella were isolated from few pastries in this study. As indicated by Costalunga and Tondo (2002), many kinds of foods were vehicle of *Salmonella spp*. The important vehicles include meats, meat products, milk products and pastry products. They also showed that pastry products are one of the most food vehicles for outbreaks of *salmonella* spp. in Rio Grande do Sul state, during the periods of 1997 to 1999, and were responsible for 16.55 % of outbreaks.

The frequency of isolation of *Salmonella* from cream cake in this study was 6.25%. When compared to similar isolation frequencies from Ethiopian foods, this was much less than that observed in 'leb leb kitfo' (42%) in Addis ababa (Mezgebu Tegenge and Mogessie Ashenafi,1998) and also much less than *salmonella* strains isolated from chicken carcass and giblet (21.16%) from De Debre Zeit and Addis Ababa supermarkets (Bayleyegn Molla, *et al*., 2003).

The contamination might have arisen due to inadequate cleaning of apparatus used for piping the cream, contamination of cream at production level followed by inefficient pasteurization or from contaminated eggs which can be a source of contamination if followed by weak heat treatment. Food handlers who also may be carrier for *Salmonella* can be also source of contamination.

The number of *Salmonella* isolated in this study was too small to make any reasonable comparisons with other studies. However, the resistance of most isolates to Penicillin, Ampicillin and Amoxacillin and the appearance of multiple resistances against all the three antibiotics is a point of concern as these drugs are commonly used in Ethiopia to treat salmonellosis.

6. CONCLUSION AND RECOMMENDATIONS

The majority of the samples examined had high microbial load which is an indicator for contamination before or after the product is processed. This may arise from improper storage products and poor hygienic status of food handlers and ingredients. Therefore, responsibility of producers and handlers is highly needed to ensure the hygiene of the finished product.

The microflora of cream cake was dominated by Gram positive microflora in which the *Bacillus* spp. has got the lion's share. Since, there may be food poisoning species within the genus and the spores can survive the baking process and potentially germinate in warm, moist environment latter, it is important to reduce the number by appropriate handling the ingredients and products.

Though, there are large numbers of consumers of cream cake pastry product in Ethiopia, there is no strong safety policy to control the quality and safety of the product as made available to the consumer. Hence, the safety and quality regulatory agencies should develop strong pastry product safety policies to control the quality and safety of products to protect the consumer from food borne infections. In addition, quality assurance protocols should be put in place. In addition, training of handlers and producers in basic principles of hygiene contribute to an integrated solution of the problem of food-borne diseases. It also urged to establish various approaches to enhance consumer awareness in the product safety, including of food safety education.

This study indicated the potential of cream cakes as source of multiple antimicrobial resistant *Salmonella* which would make treatment with some commonly used drugs difficult.

Benefits of research:

Finding of this study have shown possible health hazards related to consumption of cream cakes. Identification of these hazards would help health officials to pay attention to safety issues regarding cream cake made available to consumers. It will also contribute to awareness of consumers and producers about safety issues related to consumption of cream cakes. It may encourage producers to follow strict hygiene procedures during preparation and holding of the products.

REFERENCES

Altekruse, S.F.; Cohen, M.L. and Swerdlow, D.L. (1997). Emerging Foodborne Diseases. *Emerg. Infect. Dis.* **3:** 285–293.

Andrew, J. M. (2001). BSAC standardized disk susceptibility testing method. *J. Antmicrobiol. Chemother.* **48**: 43-57.

Anunciacao, L.L.; walter, R.L.; do Carmo,L.S. and Bergdoll, M.S. (1995). Production Of Staphylococcal enterotoxin A in cream filled cake. *Int. J. Food Microbiol*. **26:**259-263.

Azokpota,P. Hounhouigan,D.J., Nago ,M.C. and Jakobsen, M (2006). Esterase and protease activities of *Bacillus* spp. from afitin, iru and sonru; three African locust bean (*Parkia biglobosa*) condiments from Benin. *Afr J. Biotechnol.* **5:**265-272

Bayleyegn Molla, Arthuro Mesfin, Daniel Alemayehu (2003).Multiple antimicrobial-resistant *Salmonella* serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa. Ethiopia. *Ethiop.J.Health Dev.***17:**131-149.

Bhatia , A. and Zahoor, S. (2007). *Staphylococcus aureus* enterotoxins: A Review. *J. Clin. Diag. Res.* **1:**188 – 197.

Balaban, N and Rasooly, A. (2000). Staphylococcal Enterotoxins. Review:Center of Food Safety and Applied Nutrition US Food and Drug Administration Washington DC , USA.

ACMSF (2006). Report on Scientific Review of the Microbiological Risks Associated with Reduction in Fat and added Sugar in Foods.

Bremer, P. J.; Fletcher G .C. and Osborne G .C. (2004). *Staphylococcus aureus*. New Zealand Institute for Crop & Food Research Limited.

Buzby, J.C. and Roberts, T., (1997). Economic costs and trade impacts of microbial foodborne illness.World Health Stat Q. 50:57– 66.

do Carmo L.S. ; Dias,R.S. Linardi,V.R. ;de Sena M.J. and dos Santos D.A (2003). An outbreak of staphylococcal food poisoning in the municipality of Passos, Mg, Brazil.. *Braz. Arch. Biol. Technol*. **46:**581-586.

Castellanni, A.G.(1953). Inhibiting effect of amino acid and related compounds upon the growth of enterotoxogenic micrococci in cream pastery. American Institute of Baking, Chicago11,Illinois.

Costalunga, S. and Tondo, E.C. (2002). Salmonellosis in Rio Grande do Sul, Brazil, 1997 to 1999. *Braz. J. Microbiol.,* **33:**38-43.

Coughlin, F. E and Johnson, B. (1941). Gastroenteritis outbreaks from cream filled pastry. *Ame. J. pub. Health* **31**:245-250.

Dogan, I.S.; Javidipour, I. and kin Akan, T. (2007). Effects of inter-esterified palm and cottonseed oil blends on cake quality. *Int. J. Food Sci. Technol.* **42:**157-164.

Dolce, A. J. (1941). Food infection. *Amer. J. Nursing* **41:**682-684.

ECHCPD (2003). Report on Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on Staphylococcal Enterotoxins in Milk Products, Particularly Cheeses.

EHNRI(2003). Report on Prospects and Challenges of Food control system in Ethiopia.

El-Diasty, E.M. and Salem, R.M. (2007). Incidence of Lipolytic and Proteolytic Fungi in some milk Products and their public health significance *J. Appl. Sci. Res*, **3:** 684-1688.

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

Guynot, M. E.; Marin, S.; Sanchis, V.; and Ramos, A. J. (2003). Modified atmosphere packaging for prevention of mold spoilage of bakery products with different pH and water activity levels. *J. Food Prot*. **66:**1864–1872.

Hardy , A.V. (1942). Acute Diarrhea. *The Amer. J. of Nursing*. **42:**512-515.

http://www.foodmeseum.com/excake2.html.we all eat: cake. Accessed on June, 19/2008

Hugh, R. and Leifson, E. (1953). The Taxonomic Significance of Fermentative Versus Oxidative Metabolism of Carbohydrates by Various Gram Negative Bacteria Stritch School of Medicine and Graduate School, Loyola University, Chicago, Illinois .

ICMSF (2001). Guidelines for the microbiological examination of ready - to - eat foods

Jay, J.M. (1996). *Modern Food Microbiology* 4th edn.,CBS publishers and distributers, New Delhi.

Kerouanton, A.; Hennekinne, J.A.;Letertre .C.; Petit,C.L.;Chesneau,O.; Brisabois ,A and De Buyser,M.L. (2007) . Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France. *Int.J. Food Microbiol*. **115 :**369–375

McCoy J.H. (1975). Trends in *Salmonella* food poisoning in England and Wales 1941-72. *J. Hyg.***74:** 271-282.

Mezgebu Tegegne and Mogessie Ashenafi (1998).Microbial load and incidence of *Salmonella* spp. in 'kitfo', a traditional Ethiopian spiced, minced meat dish. *Ethiop. J. Health Dev.* **12:**135-140.

Needham, R. B. (2004). Early detection and differentiation of microbial spoilage of bread using electronic nose technology. University of Cranfield Institute of BioScience and Technology, PhD Thesis.

Oh, S.; Lee, N.; Cho, Y.; Shin, D.; Choi, S.Y. and Koo, M. Occurrence of Toxigenic *Staphylococcus aureus* in ready-to-eat food in Korea. *J. Food Prot*. **70:**1153- 1158.

Pereira M.; Carmo, L ; Santos, J and Bergdoll, M. (1994). Staphylococcal food poisoning from cream-filled cakes in a metropolitan area of South-Eastern Brazil. *Rev.de Saude Publica* **28:**406-409.

Reij, M.W. and Den Aantrekker , E.D (2004) Recontamination as a source of pathogens in processed foods .Review article. *Int. J.Food Microbiol*. **91:** 1 - 11.

Richter, K.S., Dorneanu, E., Eskridge, K.M., and Rao, C.S. (1993). Microbiological quality of flours. *Cereal Foods World,* **38:**367-.374.

Smith,J.P.; Daifas, D.P. ;El-Khoury,W. ;Koukoutsis,J. and El-Khoury,A (2004). Shelf life and safety concerns of bakery products—A Review. *J. Food Scie. Nutri*. **44:**19–55.

Sorokulova, I.B.; Reva1, O.N.; Smirnov, V.V.; Pinchuk1, I.V.; Lapa. S.V. and Urdaci1, M.C. (2003).Genetic diversity and involvement in bread spoilage of Bacillus strains isolated from flour and ropy bread. *Lett. Appl. Microbiol*. **37:** 169–173.

Stephens, N.; Sault, C. Firestone, S.; Lightfoot, D. and Cameron Bell, B. (2007). Large outbreaks of *Salmonella Typhimurium* Phage Type 135 infections associated with the consumption of products containing raw egg in Tasmania .*Commun. Dis. Intell*. **31:**118–124.

Stewart,C. M; Cole, M. B.and Schaffner, D. W. (2003). Managing the risk of staphylococcal food poisoning from cream-filled baked goods to meet food safety objective. *J. Food Protect*. **66:** 1310–1325.

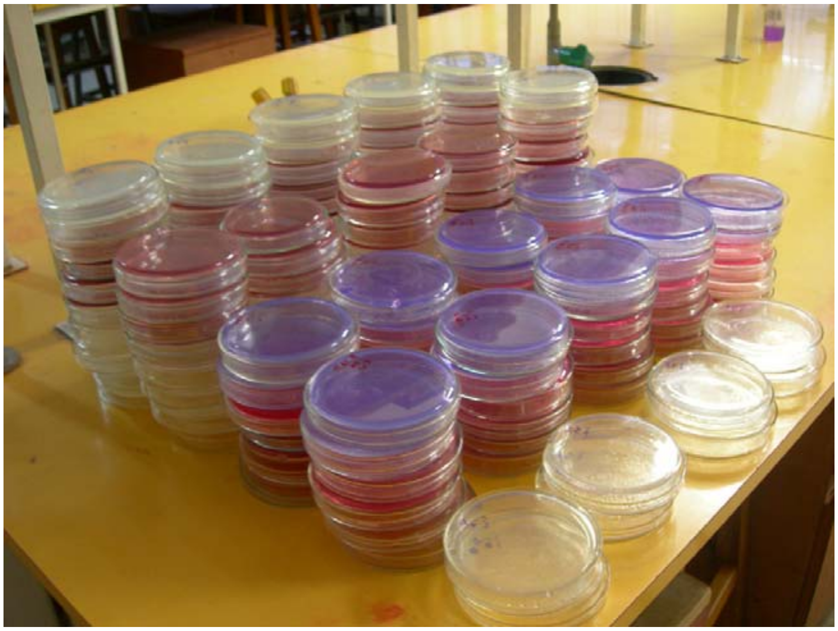
Uhitil,S.; Jaksic,S; Petrak,T.; Medic,H. and Gumhalter-Karolyi,L.(2004). Prevalence of *Listeria monocytogenes* and other *Listeria* spp. in cakes in Croatia. *J. Food Cont*. **15:**213-216.

Wyatt, C.J. and Guy, V.H. (1981). Incidence and growth of *Bacillus cereus* in retail pumpkin pies. *J. Food Prot*.**44:**422.

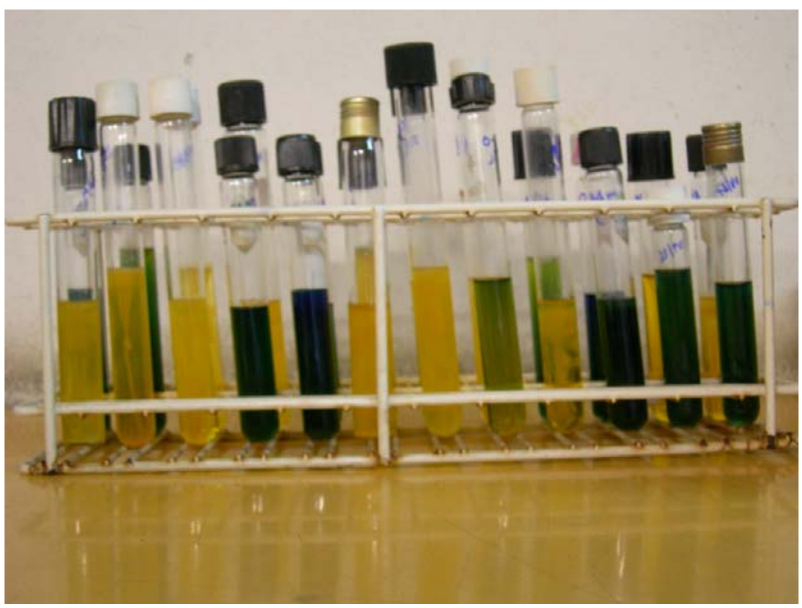
WHO (2001). Background paper: Developing a food safety strategy. WHO Strategic Planning Meeting, Geneva.

WHO (2007). Food safety and health: a strategy for the WHO African region. WHO regional officer for Africa

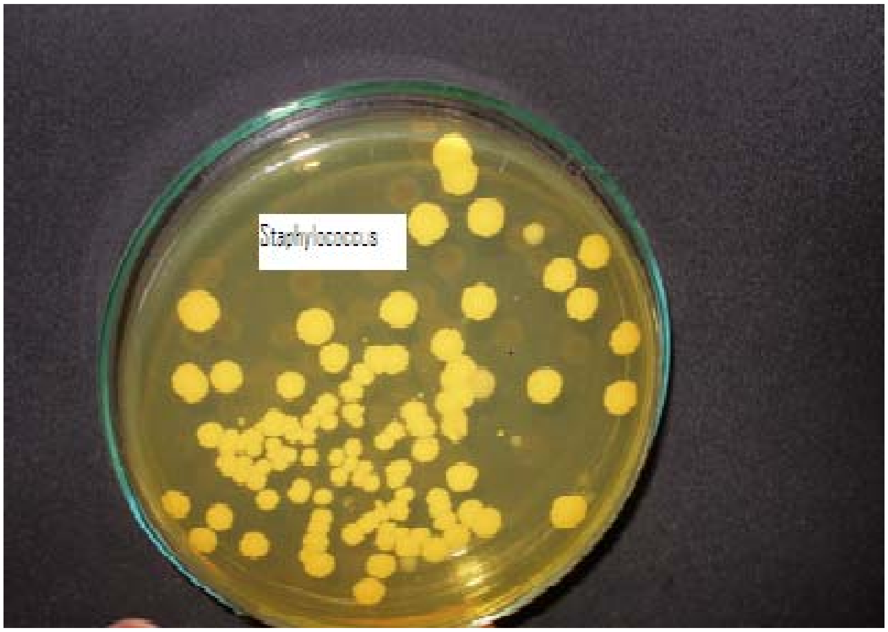
APPENDICES



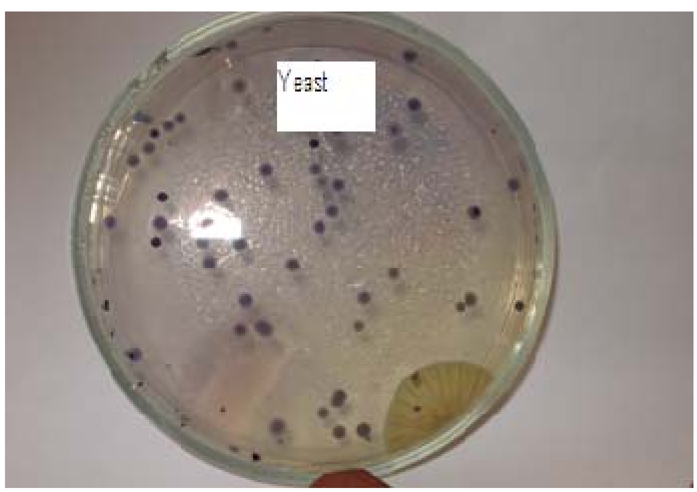
Appendix 1. All media (PCA,VRBGA,VRBA,YGC,MSA) used for plating microorganisms



Appendix 2. Test tube showing Oxidation fermentation (O/F) test

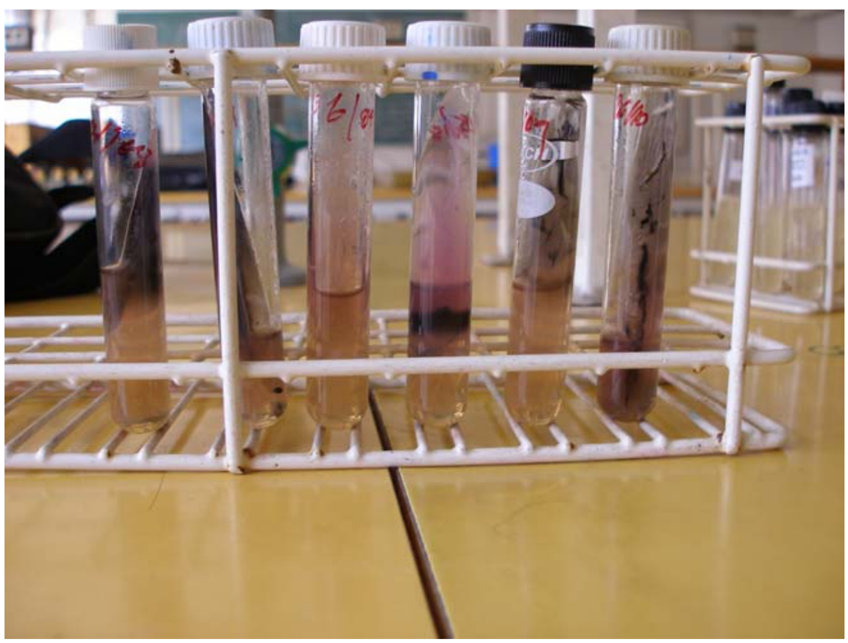


Appendix 3. Plate showing the growth of Staphylococci on Mannitol salt agar



Appendix 4. Plate showing the growth of yeast on yeast extracts glucose chloramphenicol bromophenol blue

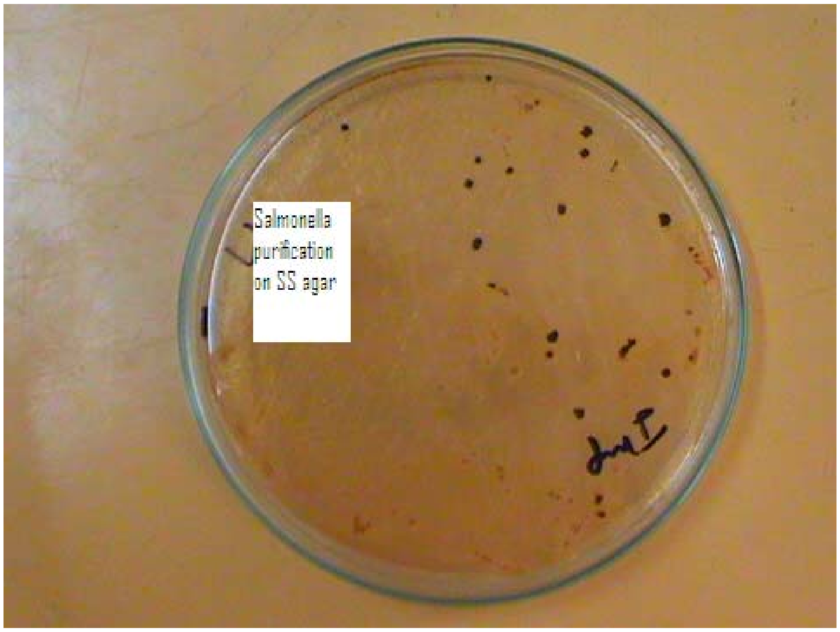
Appendix 5.Test tube showing the growth of microorganisms in secondary enrichment broths



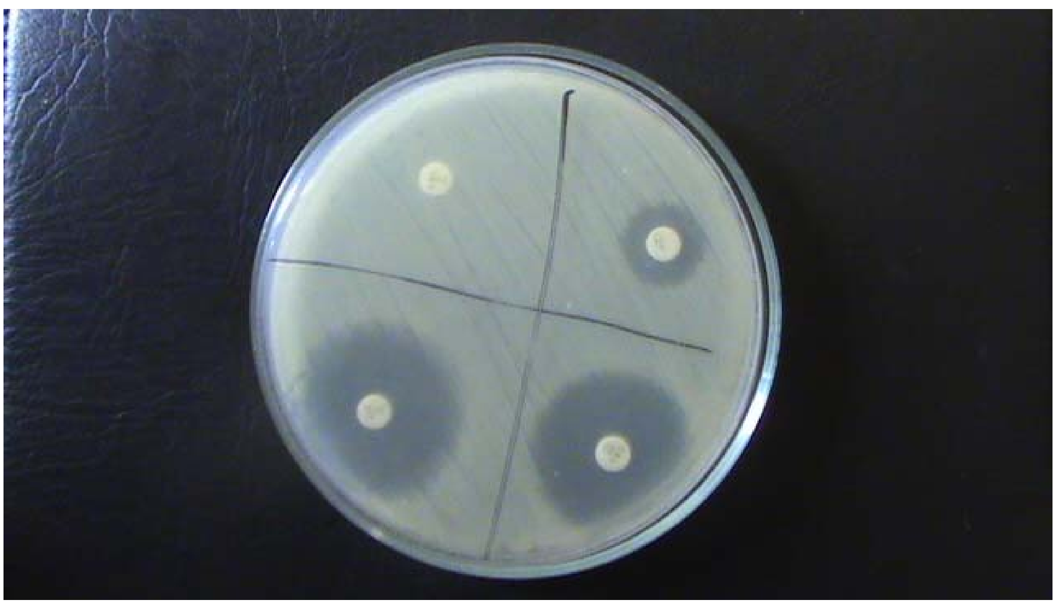
Appendix 6.Test tube showing presumptive test done for *salmonella*

Appendix 7. Bichemical test for *Salmonella* using Lysine iron agar, Triple sugar agar, Urea agar and Simmon’s citrate agar

Appendix 8. Illustration of amylolytic and non- amylolytic microbial isolates from cream cake on a starch agar plate. The isolates with clear zone around them were capable of utilizing starch.



Appendix 9.*Salmonella* purification by restreaking on SS agar



Appendix 10. Antibiotic sensitivity test for *salmonella* isolated from cream cakes