2018-01-10

THE BACTERIOLOGICAL QUALITY AND SAFETY OF HOMEMADE YOGHURT (ERGO) FROM RESTURANTS AND CAFTERIAS IN BAHIR DAR TOWN

Haregua, eshome

BAHIR DAR UNIVERSITY

COLLEGE OF SCIENCE

THE BACTERIOLOGICAL QUALITY AND SAFETY OF HOMEMADE YOGHURT (ERGO) FROM RESTURANTS AND CAFTERIAS IN BAHIR DAR TOWN

By

Haregua Teshome

AUGUST, 2014

THE BACTERIOLOGICAL QUALITY AND SAFETY OF HOMEMADE YOGHURT

(ERGO) FROM RESTURANTS AND CAFTERIAS

IN BAHIR DAR TOWN

By

Haregua Teshome

Advisor: Dr. Mulugeta Kibret

A THESIS IS SUBMITED TO THE DEPARTMENT OF BIOLOGY PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY (APPLIED MICROBIOLOGY)

August, 2014

Bahir Dar University

Bahir Dar, Ethiopia

APPROVAL SHEET

As a thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance, by Haregua Tehome Woldegebreal entitled "The Bacteriological Quality and Safety of Homemade Yoghurt (Ergo) from Resturants and Cafterias in Bahir Dar town''. I recommended the paper to be submitted as fulfilling the requirement for the Degree of Master of Science in Applied Microbiology.

---------- ---------- ----------

Advisor Signature Date

As members of the board of examiners for the MSc thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Haregua Teshome and examined the candidate. We recommended the thesis to be accepted as Fulfillment for the Requirements of the Degree of Master of Science in Biology (Applied Microbiology).

---------- ---------- ----------

Chairperson Signature Date

---------- ---------- ----------

Internal Examiner Signature Date

---------- ---------- ----------

External Examiner Signature Date

DECLARATION

The research work described in this thesis was carried out in Bahir Dar Town 2014 under supervision of Dr. Mulugeta Kibret.

I the undersigned, M.Sc. student declare that this thesis is my original work in partial fulfillment for the requirements for the degree of Master of Science in Applied Microbiology. All the sources of the materials used for this thesis and all people and institutions who gave support for thesis work are fully acknowledged.

Name: Haregua Teshome

Signature----------

Date of submission----------

This work has been done under my supervision.

Name----------

Signature----------

Date----------

ABSTRACT

Homemade yoghurt is the most popular type of fermented milk in Ethiopia. It provides nutrients to all ages of the human race. Similarly it is a good growth medium for spoilage and pathogenic microorganisms. Food borne illness associated with homemade yoghurt is attributed primarily to contamination by food handlers followed by poor sanitation of utensils and environment during manufacture and storage. The objective of this study was to evaluate the bacteriological quality and safety of homemade yoghurt and to assess hygiene and handling practice of vendors in restaurants and cafeterias of Bahir Dar town. A cross- sectional study was done from May to June, 2014. A total of 30 homemade samples were purposively collected from restaurants and cafeterias in Bahir Dar town for bacteriological analysis according to the standard procedures such as aerobic measophilic count, total coliform count, fecal coliform count and isolation of *Salmonella.* The hygienic and handling practices of vendors were assessed using observation checklist and interviews. For the aerobic mesophilic count all of the samples had unsatisfactory microbial level with a mean of 7.44 log cfu/ml. The total coliform and fecal coliform of all of the samples were in the acceptable and unsatisfactory microbial level with a mean of 3.45 log cfu/ml and 3.13 cfu/ml, respectively. In addition, *Salmonella* detected in 9 (30%) homemade yoghurt samples were unacceptable for consumption. Therefore the established level of unsatisfactory, acceptable and unacceptable microbial level with poor sanitary conditions and poor food hygiene practices of handlers required periodic sanitary-hygienic evaluation and inspection of catering establishments and educational programs targeted at improving the attitude of food handler should be strengthened to reduce public health hazards.

Keywords and phrases: Bacteriological analysis, food handlers, homemade yoghurt, hygiene, practices.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Almighty God for his endless blessings in all my life. I would like to extend my gratitude to my advisor Dr. Mulugeta Kibret for his valuable support, encouragement, supervision throughout this research work. And also for providing me his laboratory with all its well equipped facilities and materials. I am grateful to his guidance, fruitful comments and suggestions throughout the study period I have spent at Bahir Dar University.

My sincere appreciation should go to Belete Assefa for their guidance while conducting laboratory activities. I am also thankful to all of my friends for their support throughout my work.

I would like to thank College of Science, Bahir Dar University for giving permission to carry out laboratory work.

Bahir Dar town Restaurants, cafeterias owners and vendors for their willingness to provide homemade yoghurt samples and datas.

Finally, I would like to thank my families for their endless support and encouragement.

TABLE OF CONTENTS **PAGE**

LIST OF TABLES **PAGE**

LIST OF TABLES IN APPENDICES **PAGE**

LIST OF ABBREVIATIONS

ADASC Australian Dairy Authorities Standards

AMC Aerobic Mesophilic Count

ANOVA Analysis of Variance

ANRS Amhara National Regional State

EHNRI Ethiopian Health and Nutrition Research Institute

FAO Food and Agricultural Organization

FDRE Federal Democratic Republc of Ethiopia

FSAI Food Authority of Ireland

HACCP Hazard Analysis Critical Control Point

HPA Health Protection Agency

LAB Lactic Acid Bacteria

MMC Mesophilic Microorganisms Count

NSW New South Wales

PCA Plate Count Agar

VRBA Violate Red Bile Agar

WHO World Health Organization

1. INTRODUCTION

The consumption of fermented milks by man dates from the beginning of civilization (McKinley, 2005). It is accepted that the initial consumption of fermented milk products, such as yoghurt, butter and cheese, occurred around the time as they were recognized as effective means of prolonging the shelf-life of milk (Rodrigues *et al.,* 2010). Fermented milk products are known for their taste, nutritive value and therapeutic properties (Huyam *et al.,* 2013).

Yoghurt is the most popular type of fermented milk product (EI-Malt *et al*., 2013). It is a vast food category with a long and rich history. Like other fermented foods, such as wine and cheese, yoghurt was probably discovered by accident, and its exact origins are unknown. However, its early history is likely interwoven with the general history of agriculture (Kosikowski and Mistry, 1997). It is fermented by lactic acid producing bacteria*, Streptococcus thermophilus* and *Lactobacillus bulgaricus* or some additional bacteria having mutual complementing metabolism. The fermentation of lactose by lactic acid bacteria (LAB) results in the production of lactic acid, carbon dioxide, acetic acid, diacetyl, acetaldehyde and several other components giving a characteristic flavor to yoghurt (Ahmed *et al*., 2013) . It is a way to preserve food products, to enhance nutritive value, to destroy undesirable factors, to make a safe product, to improve the appearance and test of some foods, and to reduce the energy required for cooking (Deslegn Amenu, 2013).

Yoghurt plays an important role in human nutrition throughout the world (Abebe Bereda *et al.,* 2014). In Ethiopia, a considerable proportion of milk is consumed in the fermented form. The fermented product has different vernacular names such as *ergo*, *ititu*, *geinto* or *meomata* among the Amhara, Oromo, Sidama, or Wolayta people, respectively (Mogessie Ashenafi, 2006). “Ergo” is a traditional Ethiopian fermented milk product, which has some resemblance to yoghurt. It has thick, smooth and of uniform appearance. It has white milky color when prepared carefully. And a primary sour milk product from which other products may be processed (Almaz Gonfa *et al.,* 2001). In most cases, household preparation of “Ergo” requires one way incubation at ambient temperatures.

The milk coagulates within 24 hrs and preferably consumed at that time due to its good flavor. But longer keeping is not advantageous because further drop in pH will result in increased wheying off, which in turn results in loss of protein as Whey (Mogessie Ashenafi, 2006). It is the most popular dairy product throughout the country which is consumed by every member of the family and considered as spatial food which serves as a basis for further processing the milk in to different dairy products. It also used as nutritional support to sick peoples, children, pregnant and lactating mothers of the family (O'Conner, 1994).

Today most fermented food products in developed countries are produced commercially in large quantities though standardized and well controlled production processes. This usually occurs through fermentation which is initiated by adding defined starter cultures and results in high quality end-products which are consistently safe for consumption (Caplice and Fitzgerald, 1999).

However, in Africa fermented foods are still frequently prepared in small quantities using traditional methods by rural communities through spontaneous fermentation or by adding a small amount of previously fermented product as a starter (Oyewole, 1997). Spontaneous fermentation can occur due to microbes inherent in the raw milk or by microbes from the environment or preparation equipment (Oyewole, 1997; Ameha Kebede *et al*., 2007). The characteristics of these products are influenced by the quality and the type of raw milk used, the production methods followed and the regional climatic conditions (Mensah, 1997; Wouters *et al*., 2002). During the preparation of traditionally fermented milks, good hygienic practises are often neglected and, therefore, these products are often of poor quality and spoilage microbes can be present (Bille *et al*., 2007; Aloys and Angeline, 2009).

Although the fermenting bacteria have shown to provide measurable defense against food borne pathogens, a broad spectrum of microbial pathogens can contaminate human food and cause illness after they or their toxins are consumed. These include a variety of enteric bacteria. During past decades, microorganisms such as *Staphylococcus aureus, Salmonella* spp., *Escherichia coli* O157:H7*, Shigella* spp., *Listeria monocytogens* and

Yersina enterocolitica, were reported as the most common food borne pathogens that are present in many foods and able to survive in milk and fermented milk products (Tekinsen and Ozdemir, 2006).

Homemade yoghurt contamination depends on factors that are difficult to quantify, such as the initial level of contamination, which in turn depends on local conditions, level of hygiene and sanitation, and the resulting degree of acidity. Therefore, fermentation cannot replace observing the principles of food hygiene or food safety and minimizing the risk of contamination. This is particularly important since some pathogens may be acid resistant and may consequently survive the fermentation process (Motarjemi and Nout, 1996). Little contamination may deteriorate the quality of yoghurt and may have very negative effects on consumer health (Ahmed *et al*., 2013). Nowadays, microbial analyses are critical for the assessment of quality and safety, with standards and specifications, and regulatory compliance (Vasavanda, 1993). Although the production of yoghurt is very traditional procedure, post fermentation handling is very critical to induce desirable properties to the product (Skiver *et al.,* 1999).

In Ethiopia Zelalem Yilma *et al*. (2007) studied occurrence and distribution of species of enterobacteriaceae in Ethiopian traditional dairy products and reported that *Enterobacter, Esherichia coli, Klebsiella, Seratia* and *Salmonella* were isolated from ergo (a traditional Ethiopian fermented milk product) sample, also reported 20% of ergo sample might have access to the product though post processing contamination like cleaning water or manipulating personnel. Almaz Gonfa *et al.* (1999) studied the microorganisms involved in the fermentation of ergo and reported that *Lactococcus, Streptococcus, Leuconostoc* and *Lactobacillus* were responsible for the souring process of ergo. They also detected fairly high numbers of micrococci, coliforms, aerobic mesophilic bacteria and yeast. For the development of microorganisms during ergo fermentation in raw that were collected from eight dairy farms in Awassa, aerobic mesophilic counts, coliform counts, Lactic acid bacteria and yeasts were detected (Mogessie Ashenafi, 1995).

There is few published study carried out by (Asaminew Tassew and Eyassu Seifu, 2011) to evaluate microbial quality of raw cow's milk taken at different sampling points from

farmers and dairy cooperatives in Bahir Dar Zuria and Mecha district. However, no published study has been done on the bacteriological quality of homemade yoghurt and hygiene and sanitation practices of the producers. The aim of this study was to analyze the bacteriological quality and safety of homemade yoghurt retailed in restaurants and cafeterias and to assess the hygiene-sanitation practices of the producers in Bahir Dar town.

Objectives of the study

General objective

To evaluate the bacteriological quality and safety of homemade yoghurt in restaurants and cafeterias and to assess the hygiene-sanitation practices of the producers in Bahir Dar town.

Specific objectives

1. To investigate the bacteriological quality of homemade yoghurt with respect to aerobic mesophilic count, coliforms and fecal coliforms
2. To isolate *Salmonella* from homemade yoghurt
3. To assess food handling and hygiene across the service provider

2. LITERATURE REVIEW

2.1. African traditional fermented milks

Fermentation is one of the oldest methods of food processing. Bread, beer, wine, yoghurt and cheese originated long before Christ. It is an attractive technique because it is low cost and low technology and it can be easily carried out at the household level (Adams and Nout, 2001). Traditional food fermentation is a relatively efficient, low energy preservation process which increases the shelf life and decreases the need for refrigeration or other form of food preservation methods. It is therefore a highly appropriate technique for use in developing countries and remote areas where access to sophisticated equipment is limited. Fermented foods are popular throughout the world and in some regions make a significant contribution to the diet of millions of individuals (Campbell-platt, 1994**).**

Fermented milk produced in the lower Egypt, farmers put fresh milk in earthenware pots and leave it undisturbed in a warm place until the cream rises and lower partially skimmed milk coagulates. The cream layer is removed and whipped by hand to butter while the remaining sour milk, often called 'Laban Rayeb' is either consumed as it is or is converted to a soft acid cheese - 'Karish cheese' (El-Gendy, 1983). Amongst the major fermented milk products of Sudan is *Rob*. It is mainly produced from surplus milk of the rainy season by nomadic tribes. During this season the housewife turns as much milk (about 80 %) into *rob* each evening.

Another example of traditionally fermented milk is *Nono*, produced and consumed mainly by the 'Fulani', a nomadic cattle rearing tribe, in Nigeria (Atanda and Ikenebomeh**,** 1991). It is domestically prepared by naturally fermenting cow milk (or occasionally goat's milk). The cream that collects at the top of the container is Fulani butter, which is a by-product, and the remaining milk in the container is *nono*.

In South Africa, traditional fermented milks, *Maas* and *Inkomasi*, were described by Keller and Jordan (1990). The two products are traditionally produced in clay pots and calabash which are used repeatedly. Bacteria present on the inner surface of the container were presumed to be responsible for the fermentation of the milk. Feresu and Muzondo

(1990) have mentioned the presence of similar fermented milk, such as *amasi* in Zimbabwe. Amasi is produced by leaving fresh raw bovine milk to ferment naturally at ambient temperature in earthenware pots or any other suitable containers (Feresu and Muzondo 1990; Mutukumira *et al*., 1995). The microorganisms inherent in the milk, the container and the surrounding air are assumed to ferment the milk within 1-3 days depending onthe ambient temperature.

In Ethiopia, Ergo, the most important traditional milk product, which is similar to yoghurt and prepared by "spontaneous" fermentation, commonly initiated by either "back slopping" or by repeated use of the same utensil (Almaz Gonfa *et al.,* 2001). Even though the composition and microbiology of fermented milks were not understood, their beneficial effects over fresh milk were recognized in Europe, Asia and Africa (Lefoka, 2009).

2.2. Nutritional benefits of yoghurt

Yoghurt is more nutritious than many other fermented milk products because it contains a high level of milk solids in addition to nutrients developed during the fermentation process. It is an excellent source of protein, calcium, potassium, phosphorous, riboflavin (vitamin B2), thiamin (vitamin B1), Vitamin A, Vitamin D and Vitamin B12, and a valuable source, niacin, magnesium and zinc (Amakoromo, 2012).

Yoghurt contains "probiotic" cultures, e.g. *Lactobacilli* and which are currently among the best known examples of "functional food". Functional foods are described as foods claimed to have a positive effect on health (Marcos *et al*., 2004).

Consumption of fermented- milk products is associated with several types of human health benefits partly because of their content of lactic acid bacteria. A wide range of other health benefits, including Improved lactose digestion, diarrhea prevention, immune system modulation and serum cholesterol reduction, have been qualified to fermented milk consumption (Mckinley, 2005). Milk and milk products in addition to being a nutritious for humans, it provides a favorable environment for the growth of microorganisms (Abebe Bereda *et al.,* 2014).

2.3 Food borne diseases

The increase in urban population during the present century and improvements in methods of milk preservation have led to large scale transportation of milk from the producer to the consumer areas (Linton, 1982). Raw milk collection and its transportation to the processing centers present a number of technical, economical and organizational problems in most developing countries in tropical regions. In less developed areas especially in hot tropics high quality of safe product is most important but not easily accomplished (Abebe Bereda *et al*., 2014) because traditional milk fermentation did not take place in controlled systems or sterilized conditions; as a result contamination with spoilage and pathogenic bacteria would normally occur. This caused fermented milks to become major vehicles of transmission for many foodborne pathogens (Mamajoro, 2009).

The pathogens can inter from different sources. They may originate on the farm from the environment or milking equipment or in processing plants from equipment, handler, or from the air and causes food borne diseases (Dogan and Boor, 2003).

2.4 Bacterial pathogens in homemade yoghurt

The significance of milk in human nutrition is considered as the best, ideal and complete food for all age groups and an excellent source of calcium, phosphorus and magnesium. However, in spite of this, milk can also not only as a potential vehicle for transmission of some pathogens but allows growing, multiply and producing toxins. A variety of pathogenic organisms may gain access in to milk and milk products from different sources and cause different types of food borne illnesses. Milk and milk products may carry toxic metabolites of different organisms growing in it. Ingestion of such products, contaminated with these metabolites, cause food poisoning. On the other hand the ingestion of viable pathogenic bacteria along with the food product leads to food borne infection. Some time these organisms undergo lysis in the gastro intestinal tract and liberate toxic substances from inside the cells which are detrimental to the health of the consumers (Aneja *et al.,* 2002).

Milk handling procedures on the dairy farms may introduce pathogenic microorganisms in to the milk. Milk is an excellent growth medium and when stored improperly will allow the rapid proliferation of pathogens. A recent survey by (Jayarao, *et al*., 2006) identified several food borne pathogenic bacteria, including *Campylobacter jejuni, Escherichia coli, Listeria monocytogens, Salmonella serovars and Yersinia enterocolitica* associated with raw milk.

Microorganisms may contaminate milk at various stages of milk procurement, processing and distribution. The health of the cow and its environment, improperly cleaned and sanitized milk handling equipment, and workers who milk cows come in contact with milk due to a number of reasons could serve as sources of microbial contamination of milk .Use of non potable water may also cause entry of pathogens in to milk. It is known that tropical conditions which have a hot, humid climate for much of the year are ideal for quick milk deterioration so pose particular problems because the temperature is ideal for growth and multiplication of many bacteria (Gilmour, 1999; Godefay and Molla, 2000). Although milk is known to posses several antimicrobial systems, bacterial numbers will double in less than 3 hours in unchilled milk. The rate of microbial growth will depend on initial numbers and the temperature at which milk is held after milking and thereafter (Kurwijila *et al*., 1992).

Lack of refrigeration facilities at the farm and household level, with high ambient temperature implies that raw milk will easily be spoiled during storage and transportation (Gilmour, 1999; Ombui *et al*., 1995; Kurwijila *et al*., 1992). Diseases that commonly spread from the milk to human beings are tuberculosis, brucellosis, salmonellosis, listeriosis, campylobacteriosis, yersinoses, and other bacterial pathogens transmitted to humans include *streptococcus agalactaciae*, *staphylococcus aures* and *Escherichia coli* (Hahn, 1996).

Milk in most places in Ethiopia is consumed raw. Milk products such as yoghurt and butter are also produced using raw milk as a starting material. Hence, there exists the possibility of consuming milk, which has been contaminated with disease causing organisms (Mehari, 1998

Food- borne pathogens in yoghurt and other dairy products are supported by their ability to adapt to the environmental factors. During fermentation, yoghurt bacteria metabolize

lactose to produce lactic acid and hence a low pH. Many pathogens are capable to adapt and survive this environmental stress. *Salmonella* species and *Staphylococcus aureus* are known to adapt to acidic environments and this promotes their survival in dairy products (Pazakova *et al.,* 1997). Therefore, it is preferably consumed soon after (24h) fermentation, because homemade yoghurt can be a potential health hazard if prepared from milk contaminated with *E. coli* O157:H7. So, despite the general assumption that low pH in homemade yoghurt control the proliferation of undesirable microorganisms, the dangers of listeriosis or salmonellosis from fresh yoghurt must not be underestimated (Mogessie Ashenafi, 2006).

Salmonella typhimurium, E. coli O157:H7 and *Staphylococcus aureus* can survive and multiply during fermentation of kefir, since these pathogens are resistant to acidic conditions, in the contamination of milk used for Kefir, they can presumably survive and cause food borne illnesses (Karagozu, 2007). The study done by, Mogessie Ashenafi, 1992; determine the possibility of contamination of milk by food-borne pathogens from various sources and the fate of pathogenic microorganism such as, *Salmonella* spp, *Bacillus cereus, Staphylococcus aureus* and *Listeria monocytogenes* during the souring of milk in to Ergo showed that, all the test pathogens grow to levels as high as 10^{7}-10^{8} cfu/ml within 12 hours in fermenting milk. The duration of inhibition of pathogenic microorganism by lactic acid bacteria is vary from one organism to the other. For example, *Salmonella typhimurium* and *Salmonella enteritidis* were inhibited between 48 and 60 hours of fermentation of milk in non-smoked and smoked containers, respectively. It was suggested that the synergistic effect of pH, acids and container smoking were important in the complete inhibition of the test organisms (Mogessie Ashenafi, 1992).

2.4.1 The genus *Salmonella*

2.4.1.1 General characteristics of *Salmonella*

Salmonella is a genus of the Enterobacteriaceae family (Lefoka, 2009). It is a group of closely related gram negative, non- sporulating, facultative anaerobic, motile, rod shaped bacteria. They are mesophilic with (5-46 ^{o}C) with the optimum growth range of 35-43 ^{o}C and a_w 0.94 (Ray and Bhunia, 2008). Growth is slow at temperature below 10 ^{o}C and most strains do not grow at temperature less than 7 ^{o}C. *Salmonella* species has a pH range for growth of 4 to 9 with an optimum of 7 to 7.5. It is between 2-5 micrometer long and 0.7-15 micrometer in diameter and has flagella which are tail like projections made of proteins which help the bacteria to move (Meerberg and Kijlstra, 2007).

The genus *Salmonella* consists of only two main spp. *Salmonella bongori* and *Salmonella enterica*. *Salmonella enterica* is divided in to six sub spp (sub spp *enteric*, Sub spp *salamae*, sub spp *arizonae*, sub spp *diarizonae*, sub spp *houtenae* and sub spp *indica*). More than 99% of *Salmonella* strains causing human infections belong to *Salmonella enterica* sub species *enteric* (Schere and Miller; 2001). They are non fastidious as they can multiply under various environmental condition outside of the living hosts. Although the bacteria cannot multiply outside of the host digestive tract but they can live for several weeks in water and several years in soil when there is favorable humidity, pH and temperature condition (Deriba Multa and Mogessie Ashenafi, 2001).

Many strains of salmonella can cause food borne illness in humans, and all strains exhibit the same symptoms such as gastroenteritis (vomiting and diarrhea).Pasteurization destroys the salmonella organism (USDA,1981).

2.4.1.2 Sources of *Salmonella*

Salmonella is ubiquitous in the environment originating from the gastrointestinal tracts of domesticated and wild animals and can be present without causing apparent illness. Most infections result from the ingestion of foods of animal origin contaminated with *Salmonella* spp such as beef, chicken, turkey pork, eggs and milk and other ready to eat food items are also sources of *Salmonella* (D'Aoust, 1995; Jacob, 2010).

Foods of animal origin particularly meat, poultry, egg, milk and milk products are considered to be the primary source of human salmonellosis (Acha and Szyfers, 2001). Most of these food products become contaminated during slaughter, processing in contaminated environment and because of faulty in transport, handling, storage or preparation.

Salmonella can contaminate the environment and can survive in the environment for a long time. As a result this environment can serves as the source of *Salmonella* infection. After that, *Salmonella* is transmitted to vectors such as rats, flies and birds where *Salmonella* can shed in their feces for weeks and even months. Following the direct transmission, moving animals such as pig, cows and chickens act as the important risk factor for infection. These animal reservoirs are infected orally because *Salmonella* normally originates from the contaminated environment and also contaminated feed (Newell *et al*., 2010). Human get infected when eating the food or drinking the water that is contaminated with *Salmonella* through animal reservoir. Therefore infection can be occurred by eating the inappropriately handled food by infected handlers (Newell *et al.,* 2010). The presence of *Salmonella* on hands and food preparing surfaces has also been reported as resulting from handing and preparing contaminated raw foods (Hobbs and Robert, 1993).

2.4.1.3 Food borne Salmonellosis

Salmonella is globally a leading cause of food borne illness (Liyuwork Tesfaw *et al*., 2013). It is an important cause of gastrointestinal illness called salmonellosis in humans. (Cross *et al.,* 1889; Smith, 1994; Baumler *et al*., 2000). Based on information provided by the Centers of Disease Control (CDC), numerous food products are associated with the contamination of *Salmonella*, although, products from animal's origin are mainly common. The true incidence of salmonellosis is both in humans and animals are difficult to evaluate because of lack of an epidemiological surveillance system in place, which is particularly true in developing countries. However in countries with a reporting system, the number of out breaks particularly in humans has increased considerably in recent

years (D'aoust, 1994). The exact cause of the predominance of these *Salmonella* serotypes is not yet clearly understood (CDC, 2007).

In developing countries, street foods in particular have been reported to be contaminated with *Salmonella* species and have been implicated in few outbreaks of foodborne salmonellosis (Mankee *et al.,* 2003). Now a day numerous outbreaks of gastroenteritis especially outbreaks of salmonellosis have been associated with *Salmonella* infected ready to eat food and food handlers. Salmonellosis is more common in the warmer months of the years were observed among the isolates (Deriba Muleta and Mogessie Ashenafi, 2001).

In Ethiopia recent studies made elsewhere indicated that milk and milk products are important source of *Salmonella* particularly among those raw consumers. Ubiquitous nature of *Salmonella,* unhygienic condition prevailing at the farm levels and food handlers, and habit of consuming milk and milk products in raw suggest that milk and milk pro-ducts can act as source of *Salmonella* organisms in Ethiopia. Studies shows that Considerable proportion of them might have developed resistance to antimicrobials that are commonly used in both the veterinary and public health. Such a problem might be significant in areas like Addis Ababa where consumption of milk and milk products are high, dairy supermarkets are significant, and handling of milk and milk products take several hours until they reach to consumers (Liyuwork Tesfaw *et al*., 2013).

Most food borne salmonellosis outbreaks have been implicated to foods containing eggs or poultry products. Nevertheless, there have been several outbreaks of salmonella for which milk or milk products were responsible (Vlaemynck, 1994).

2.5 Indicators of bacteriological quality of homemade yoghurt

“Indicator organism” refers to the selected surrogate markers. The main objective of using bacteria as indicators is to reflect the hygienic quality of food (FEHD, 2007). Aerobic mesophilic bacteria, enterobacteriaceae, *E. coli*, coliform and fecal coliform are some of indicator organisms.

2.5.1 Aerobic mesophilic count (AMC)

The aerobic mesophilic count is among the more popularly used nonpathogenic microbiological indicators of sanitary quality of foods (Miskimin *et al*., 1976). It is an important microbiological parameter for milk and dairy products quality and when present at high levels (higher than 10^5 cfu/ml) indicate serious deficiencies in production hygiene, where as values lower than 20,000 cfu/ml reflect good sanitary practices (Chambers, 2002). The only obligatory microbiological criterion is the mesophilic microorganisms count (MMC) that can reach to a maximum of 100 thousand per gram expressed in counts of colonies forming units (CFU) (Foltys and Kirchnerovamilk, 2006).

It is generally believed that high aerobic mesophilic counts in foods indicate greater risks of pathogens being present in consumable products. Poor fermentation of sanitation procedures or problems in process controls to which a test food has been subjected (Miskimin *et al*., 1976).

High bacterial numbers spoil the food faster and result in loss of quality. Foods which appears normal may have high AMC indicating that the food is about to spoil. In fresh products AMC indicates the effectiveness of sanitary procedures used during processing and handling and before storage of the product. A high AMC in food products that were given heat treatment may indicate that both shelf life stability and safety is affected (Jacob, 2010). Bacterial infections in humans are mostly caused by mesophilc bacteria that find their optimum growth temperature around 37^oC in the normal human body temperature (Patrica and Azanza, 2004). Some strains of common mesophilic bacteria which are not commonly associated with foodborne disease, however, may cause illness when present in excessive numbers (Aycicek *et al.,* 2006; Jacob, 2010). Many processes in the food industry and most fermentation process occur within this temperature range. This is also the temperature at which the vast majority of pathogenic bacteria function and also the temperature at which most bacteria attack our bodies. There for this presents the need for close control of bacteria action within those food industries (Jacob, 2004).

2.5.2 Coliform Count (CC)

Coliforms are commonly used indicators of sanitary quality of food and water or as a general indicator of sanitary condition in the food processing environment (Hamadan *et al.,* 2008).

Coliforms are also another typical indicator of food quality. They represent group of species from genera of *Escherichia, Enterobacter, Klebsiella, Citrobacter*, and probably *Aeromonas* and *Serratia.* They are facultative anaerobes, gram-negative, non-spore forming, rod shaped bacteria that ferment lactose (a type of sugar), producing gas and acid within 48 hours when cultured at 37^0c. Their lack of ability to form spores makes them more susceptible to destruction by environmental conditions (Environmental Fact Sheet, 2003).

Coliform count of less 100 colony \ml in yoghurt is considered to be acceptable, but count of coliform less 10 colony/ml is achievable and desirable (Boor *et al*., 1998). Coliform count greater 100 colony/ml indicate poor hygiene either during equipment cleaning or between milking with common contaminants such as blanket, compost, soil or water (Murphy and Boor, 2003). Increased counts of coliform bacteria are indicative of failure in sanitation and very high counts are dangerous to human health (Napravnlkoval *et al*., 2002). But this does not mean all food that is free from coliform are safe (Smith and Schaffner, 2004). Coliform organisms have an unfavorable influence on keeping quality of milk and milk products. Coliforms bacteria could survive during pasteurization (Havlova *et al*., 1993). Coliforms are almost always found in raw milk but with good methods of production number of coliforms can be kept very low (Boor *et al.*, 1998).

The group of coliforms may proliferate rapidly in milk and milk product at temperature above 10^0c and unless samples of fresh milk or milk products which have been stored at low temperature are examined, unsatisfactory tests may be the result of such proliferation rather than of initial contamination (Murphy and Boor, 2003).

Fecal coliform bacteria are bacteria that found in feces and it is a member of coliform bacteria, growing at 44.5^0C with in 24hrs. Coliforms from non-fecal, environmental sources are incapable of growing at this elevated temperature (ENVR431, 2008). Fecal coliforms normally reside in the intestinal tract of warm-blooded animals and are indicators of fecal contamination. Outside of a warm-blooded host, fecal coliforms are short- lived compared to the coliform bacteria that are free-living and not associated with the digestive tract of man or animals (Environmental Fact Sheet, 2003). Some of them are pathogenic and cause diseases like typhoid, dysentery and enteric fever (Salle, 2000; Reddy *et al*., 2009).

Coliform organisms contaminate raw milk from unclean milker's hands, improperly cleaned and unsanitized or faulty sterilization of raw milk utensils especially churns, milking machines, improper preparation of the cow's flecks or dirt, manure, hair dropping in to milk during milking, udder washed with unclean water, dirty towels and udder not dried before milking(Ombui *et al*., 1995).

The presence of coliform organisms in milk indicates unsanitary conditions of production, processing or storage .Hence their presence in large number in dairy products is an indication that the products are potentially hazardous to the consumers' health (Godefay and Molla, 2000).

2.6. Food Handling and Hygienic practices

It is possible to control and minimize the number of organisms present in food using good food hygienic practices in its preparation and handling or by processing the food in some way. Rules of good hygienic practices in food preparation deal broadly with their different areas which includes physical factors relating to the premises and utensils used operational factors relating to the hygienic handling of food and personal factors relating to issue of personal hygiene and training (Adams and Motajemi, 1999). The handling practices in the food preparation areas therefore provide an opportunity for cross contamination of the bacteria to ready to eat foods.

The good handling problems during purchasing and delivery include the chance that the food product is contaminated either at the food source, during delivery or during

transportation to the restaurant. The microbial analyses of the workers hand were made for some microorganism, including the aerobic mesophilic plate counts, as well as the some food pathogens (Almeida *et al*., 1995). The vendors can be carriers of pathogens like *Escherichia coli, Salmonella, Shigella*, *Campylobacter* and *S. aureus* who eventually transfer these foodborne hazards to the consumers. The hands of the food handles are the most important vehicle for the transfer of organisms from feces, nose and skin to the food (WHO, 1989). The findings that *Salmonella*, *Campylobacter* and *E. coli* can survive on finger tips and other surfaces for varying periods (Pethers *et al.*, 1971).

Food handlers often have little understanding of the risk of microbial or chemical contamination of food or how to avoid them. From some essential information on safe food handling that person should be required to know particular attention should be given to the importance of time, temperature control personal hygiene, cross contamination and the factors determining the survival and growth of pathogenic organisms in food (WHO, 1989). Food handlers should have the necessary knowledge and skills to enable them to handle food hygienically (FAO, 1997).

The composition of milk and milk products makes them good media for the outgrowth of pathogenic micro organisms. The value of 40% annual milk and dairy product losses due mainly to mishandling across five African and the Middle East countries (Kenya, Tanzania, Uganda, Ethiopia and Syria). Therefore, Food handlers should therefore, receive suitable training in the basic principles of food safety, including the hazard analysis critical control point (HACCP) system (WHO, 2004). Hazard Analysis and Critical Control Point (HACCP) in particular is employed in the identification of stages in the food chain where spoilage as well as pathogenic microorganisms can enter, survive and proliferate in the food and managing these as the key control strategy rather than relying on testing end product. In the Ethiopian smallholder context, the Critical Control Points (CCPs) where homemade yoghurt product can be contaminated are:-

1). During milk production: During milking, contamination can come from the cow, the milker, utensils used for milking, storage and filtering the milk, and the barn or the milking environment. 2). During fermentation: Milk containers used for fermentation,

wash water of poor quality used, ingredients added with the intention of improving the flavor of the final product can represent potential sources of contamination. 3). Packaging/storage of the product: Potential sources of contamination here include: product container/packaging, high keeping temperature, and poor personal hygiene of people handling the product. Reducing such losses and improving quality are effective ways of making more and safer milk available that benefits both producers and consumers. Provision of milk and milk products of good hygienic quality is desirable. Implementing the proper hygienic control of milk and milk products throughout the food chain is essential to ensure the safety and suitability of these foods for their intended use (Zelalem Yilma, 2007).

Hygienic quality control of raw milk and milk products in Ethiopia is not usually conducted on routine basis. Apart from this, door to door raw milk delivery in the urban and peri-urban areas is commonly practiced with virtually no quality control at all levels (Godefay and Molla, 2000).

3. MATERIALS AND METHODS

3.1. Description of the study area

This study was conducted in Bahir Dar town, which is the capital city of Amhara National Regional State (ANRS). Bahir Dar is located in 578 km Northwest Addis Ababa having location of $11^0$36'N latitude and $37^0$23' longitude and has an elevation of 1840m above sea level. The area of the town is about 160km^2 and there are around 256,999 people live in there (CSA, 2011). In Bahir Dar the temperature ranged from 20-34^{0}c in months from December up to April with humidity range of 47-66%. While the temperature may rise to higher value during May. This temperature and humidity range is favorable for microbial growth if there is contamination of homemade yoghurt with pathogens and may result in food-borne disease on consumers. In the town, there are restaurants, cafeterias, dairy and dairy products shops that have retailed homemade yoghurt.

3.2 Study design

A cross-sectional study was conducted from May 2014-June 2014 to assess the bacteriological quality and safety of homemade yoghurt retailed in restaurants and cafeterias in Bahir Dar town. Likewise the handling and sanitation practice of vendors in restaurants and cafeterias in Bahir Dar town was assessed.

3.3. Sample collection

To carry out the proposed research a total of 30 samples of homemade yoghurt were purposively collected from different restaurants and cafeterias found in Bahir Dar town. Samples were collected under aseptic conditions in sterile airtight sampling bottles from 5-6 hour and brought to Bahir Dar University Microbiology Laboratory Room in an ice box and kept aseptically under refrigeration at 4^{o}C for 1-2 hour until bacteriological analysis were done.

3.4. Bacteriological analysis of the collected samples

Bacteriological analyses were done according to standard procedures for the number of aerobic mesophilic bacteria, total coliform, fecal coliform and salmonella.

3.4.1. Sample preparation

One hundred fifty ml of each homemade yoghurt samples were homogenized thoroughly by shaking then from 150ml homogenized homemade yoghurt, 25ml was aseptically removed and added into 225 ml of sterile 0.85% NaCl solution in a clean 500 ml sterile flask, shaken to make 10^{-1} dilution, and then serially diluted as needed (10^{-2}, 10^{-3}…) (Richardson, 1985).

3.4.2. Enumeration of aerobic mesophilic bacteria

Serial dilutions of (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}) were made by adding 1ml dilution in to sterile test tube having 9 ml sterile physiological saline solution. One ml from each dilution was added into sterilized duplicate petridishes for each dilution by using sterile pipette. Then 15 to 20 ml of melted plate count agar (Oxoid, England) was poured to each plate. All plates were incubated in aerobic atmosphere at 37^0C for 48 hours. The numbers of colonies were counted with colony counter (Stuart Scientific Colony Counter, UK) and the result was recorded as cfu/ml of Yoghurt. The total aerobic mesophilic count of bacteria was determined using the procedure described by (Akabanda, 2010).

3.4.3. Total coliform count

Serial dilutions of (10^{-1}, 10^{-2} and 10^{-3}) were made by adding 1ml dilution in to sterile test tube having 9 ml sterile physiological saline solution. Then one ml from each dilution was added into sterilized duplicate petridishes for each dlution by using sterile pipette. Then 15 to 20 ml of melted Violate Red Bile Agar (VBRA) (Oxoid, England) was poured to each plate. Plates were incubated at 37^0c for coliform count and at 44^0c for fecal coliform count. Both coliform and fecal coliform plates will be incubated for 24h. (ISO; 4832, 1991). Purplish red colonies surrounded reddish zone of precipitated bile were

counted as coliforms (Eleanor, 2007).The numbers of colonies were counted with colony counter (Stuart Scientific Colony Counter, UK) and the result was recorded as cfu/ml of yoghurt.

3.4.4 Isolation of *Salmonella*

One ml of the homogenized yoghurt sample was pre-enriched in 9ml selenite cystine broth (SCB) (Blulux, India) under aerobic atmosphere at 37^0C for 8-18 hours. After pre-enrichment, a loop full of inoculums was streaked on Salmonella Shigella Agar (SSA) (Bhiwadi, 301019, India) and then incubated under aerobic atmosphere at 37^0C for 24 hours for isolation of *Salmonella.* After incubation, *Salmonella* colonies were selected from SSA as (colorless colonies with black centers) (Mudgil *et al.,* 2004). Two or three of typical colonies (colorless with black center on SSA) were selected and transferred to nutrient agar and incubated at 37^0c for 24 hrs. The culture from nutrient agar were streaked onto Tryptic Soya Agar (TSA) (Donwhitley, India) slant and incubated at 37^0_c for 24 hour and stored at 4^0C for further biochemical identification (Abd EI-Atty and Meshref, 2007).

Species that cannot be distinguished by their morphology and cultural characters exhibit metabolic differences. To do this a loop full of inoculums was taken from TSA suspension to carry out the following biochemical tests (David *et al*., 2006).

A) Sulfide Indole Motility medium (SIM): (Blulux, India) It was used to test hydrogen sulfide (H2S), indole production and motility of the organism. Sterile inoculating needle inserted in TSA suspension was immersed straight down and stubbed straight back into SIM and incubated at 37^0c for 24 hours. Motility was determined by the presence of diffuse growth away from the line of inoculation (cloudy medium) which is observed in the presence of motility. Production of H2S is indicated by blackening of the medium and for indole production, formation of red colored layer on the surface of SIM medium when 2-3 drops of Kovac‟s reagent was added. *Salmonella* species were motility positive, indole negative and variable for hydrogen sulfide production.

B) Glucose and lactose fermentation and gas production test: Klingler Iron Agar (KIA)/Triple sugar Iron agar (TSIA) (Oxoid, England) slant was used to determine whether the organism ferment glucose and lactose and involved in the formation of gas. The butt was stabbed and the slant was streaked with sterile inoculating loop containing a loop full of inoculums from TSA suspension and then incubated for 24 hrs at 37^0c . *Salmonella* spp. produces acid (yellow butt) due to glucose fermentation.

C) Citrate utilization test: Simmons Citrate Agar (Himedia) slant was used to check utilization of citrate as carbon source. The butt was stabbed and the slant was streaked with inoculating loop and then incubated at 37^0c for 24 hours. The butt was changed in to blue color when there is citrate utilization. *Salmonella* spp. was variable for citrate utilization. Alkaline (red) slant that indicated the absence of lactose fermentation and variable in gas production.

D) Lysine decarboxylase test: Both the slant and the butt of the agar (Himedia) were inoculated, to check whether the organism was able to produce the enzyme lysine decarboxylase or not. Most *Salmonella* spp. was positive for lysine decarboxylase test and show positive lysine decarboxylase reaction (purple /alkaline) butt and slant. But, some *Salmonella* species were negative for the enzyme production that was indicated by pale yellow butt and pink slant color.

E) Urea hydrolysis test: Urea agar base (Oxoid, England) was used to test whether the organism hydrolyzes urea or not. Urea broth was inoculated heavily over the entire surface of the broth and then incubated at 37^0c for 24 hours. Urease positive cultures produced an alkaline reaction in the medium and showed pinkish-red color formation while, urease negative organisms did not change the color of the medium, which is a pale yellowish color. *Salmonella* spp. was always urease-negative.

3.5 Assessments of the hygienic practices of venders and vending area

Observation checklist and interview was used for assessment of the sanitation conditions of the vending environment and personal hygienic practices of venders. Check lists and interviews covering topics on the personal hygiene of the food handlers and sanitation of

the vending environment were used for assessment. Assessments of the hygienic practices of venders and vending environment were done on 30 food handlers.

3.6 Data analysis

Data were analyzed through Statistical Package for Social Sciences version 20 statistical package. Descriptive statistics such as means and frequencies were used to present the findings. P-value <0.05 was considered to indicate statistically significant association.

4. RESULTS AND DISCUSSION

4.1 Aerobic mesophilic counts

In this study the aerobic mesophilic count of homemade yoghurt samples were determined. Minimum, maximum, mean and standard deviation of homemade samples from different restaurants and cafeterias were given in (Table 1). The mean aerobic mesophilic count of various homemade yoghurt samples were the ranged from 6.60 to 7.75 log cfu/ml with average count of log 7.44cfu/ml (Table 1). This finding is in agreement to study with Savadodogo, 2010; Zelalem Yilma, 2010; Fekadu Beyene, 1994; Mogessie Ashenafi, 1995 reported 5.9 log cfu/ml to8.556 log cfu/ml in Burkinafaso, 7.71 logcfu/ml in sheno, 8.6 log cfu/ml from three villages in southern Ethiopia and >9 log cfu/ml in Awassa from eight dairy farms, respectively. In contrary low range of AMB (2.76-3.88 log cfu/ml) was reported by Mourad and Nour-Eddine (2006).

4.2 Total coliform counts (TCC)

In the present study the total numbers of coliforms in homemade Yoghurt were determined. Minimum, maximum, mean and standard deviations of homemade samples from different restaurants and cafeterias were shown in (Table 1). The mean total coliform count of various homemade ergo samples were ranged from 2.60 to 3.64 log cfu/ml with a mean value of 3.45 log cfu/ml (Table 1). This finding is comparable to the study with Al-Kadamany *et al*. (2003) and Hempen *et al.* (2004) reported 3 log cfu/ml, 4 log cfu/ml, respectively. They reported that this high number of coliform can cause rapid spoilage of milk because they are able to ferment lactose with the production of acid and gas. High total coliform count also reported between log 5 cfu/ml to log 6 cfu/ml, by Farzana *et al*. (2009) and Younus *et al.* (2002), Kumbhar *et al.* (2009) and Mogessie Ashenafi, (1995), respectively. They reported that this reflects highly poor hygienic conditions and improper sanitation during making ergo.

4.3 Fecal coliform counts

In the present study the total numbers of fecal coliforms in homemade Yoghurt were determined. Minimum, maximum, mean and standard deviations of homemade yoghurt samples from different restaurants and cafterias are shown in (Table 1). The fecal coliform count ranged from 2.51 to 4.48 log cfu/ml with a mean value of 3.13 log cfu/ml. This result is lower than the results of the study with Zelalem, 2010 reported 4.51 log cfu/ml from ergo sampled Sheno to Jimma. Indicator organisms are those which serve to indicate objectionable conditions of foods such as recent or remote fecal contamination, presence of potential pathogens in the food, as well as sanitary conditions of the food processing, production and storage facilities (Abera Geyid *et al.,* 2001).

Table 1: Bacteriological Count (log cfu/ml) of homemade yoghurt from Resturants and Cafterias in Bahir Dar town, 2014 (n=30).

Indicator bacteria	Minimum	Maximum	Mean + SD
AMC	6.60	7.75	7.44±0.35
TCC	2.60	3.64	3.45±0.29
FCC	2.51	4.48	3.13±0.29

SD=Standard deviation

In this study the aerobic mesophilic bacteriological quality levels of homemade yoghurt samples from different restaurants and cafeterias were determined based on their bacterial count. Satisfactory, Acceptable and unsatisfactory levels (ADASC, 2000). All homemade yoghurt samples from restaurants and cafeterias analyzed. Thirty (100%) were unsatisfactory microbiological quality level (Table 2). This finding is above the recommended value of Australian Dairy Authorities'Standards Committee (ADASC, 2000). This High number of aerobic mesophilic counts is not in itself health risk but it indicates an overall lack of hygiene (Ray, 2004). According to Mogessie Ashenafi (2002) in most households of Ethiopia no attempt is made to control the fermentation process of

milk and products manufactured under traditional systems generally have poor qualities and do not meet the acceptable quality requirements set by various regulatory agencies.

In this study the total coliform bacteriological quality levels of homemade yoghurt samples from different restaurants and cafeterias were determined based on their bacterial count. Satisfactory, Acceptable and unsatisfactory levels of all thirty samples are given in (Table 2). All homemade yoghurt samples from restaurants and cafeterias analyzed. Thirty (100%) acceptable microbial level for consumption (Table 2). This result was above the recommended value of Food Authority of Ireland (FSAI, 2001). The presence of coliform bacteria in dairy products indicates unhygienic conditions during storage, transportation cross contamination, poor cleaning, poor temperature and time control. Even if there is absence of unsatisfactory and hazardous samples in respect to this parameter, it will not give a guarantee to the absence of contamination with human matter. It must be pointed out that they demand further attention in the future because any deterioration or incident in the production, post-process contamination and storage condition including hygienic condition will allow the transition in the area of unsatisfactory results, While coliforms are not a cause of food borne illness very high levels may probably come from contaminated water used for washing purpose (Lewis *et al.,* 2006).

In this study the fecal coliform bacteriological quality levels of homemade yoghurt samples from different restaurants and cafeterias were determined based on their bacterial count. Satisfactory, Acceptable and unsatisfactory levels of all thirty samples are given in (Table 2). All homemade yoghurt samples from restaurants and cafeterias analyzed. Thirty (100%) is unsatisfactory microbiological quality level for consumption (Table 2). This finding is above the recommended value of Food Authority of Ireland (FSAI, 2001). The existence of Fecal coliform in milk and milk products is suggestive of fecal contamination and unsatisfactory results (Zelalem Yilma, 2010).

Table 2: Bacteriological quality levels of homemade yoghurt based on bacteriological count taken from Resturants and Cafterias in Bahir Dar town, 2014 (n=30).

Indicator bacteria	Satisfactory	Acceptable	Unsatisfactory
AMC	_	_	30 (100%)
TCC	_	30(100%)	_
FCC	_	_	30 (100%)

4.4 Isolation of *Salmonella*

In this study 30 homemade yoghurt samples were used to isolate *Salmonella* (Table 3).

Table 3: *Salmonella* isolated from homemade yoghurt selling restaurants and cafeterias in Bahir Dar town, 2014 (n = 30)

Salmonella result	Frequency No (%)
Salmonella positive	9 (30)
Salmonella negative	21 (70)
Total	30 (100)

From the total 30 homemade yoghurt samples collected from restaurants and cafeterias, 9 (30%) samples were positive for *Salmonella*. The presence of *Salmonella* is beyond the acceptable guide lines set for Salmonella which is not detected in 25ml (ADAS, 2000). The detection of *Salmonella* and in many of a sample examined is regarded as potentially hazardous to consumers and is unacceptable for consumption (Cheung *et al*., 2007). Studies in Mayssoun and Nadine (2010) and Kumbhar *et al*., (2009) showed the presence of *Salmonella* may be result from poor hygiene of farms, personnel, utensils.

4. 5 Assessment of the handling practices of the food handlers and sanitary conditions of the vending environment.

4. 5. 1. Socio-demographic profile of the food handlers:

A total of 30 homemade yoghurt selling food handlers were investigated during the study. The socio-demographic data of the food handlers is presented in Table 4. All of the food handlers were females where most of them (60%) were between 21 and 30 years of age. Majority (53.3%) of the food handlers completed at least primary school and 13.3% had no formal education. Most of the food handlers (80%) were single while 20% of them were married. All of food handlers in the study have no received food hygiene training (Table 4).

Table 4: Socio- demographic profile of food handlers in Bahir Dar, Ethiopia, 2014

Parameter	Frequency	Percent
Age		
15-20	5	16.7
21-30	18	60
>30	7	23.3
Educational background		
1-8	16	53.3
8-12	10	33.3
No formal education	4	23.3
Marital status		
Married	6	20
Single	24	80
Received food hygiene training		
Yes	0	0
No	30	100

In the current study only 13.3% of the vendors interviewed illiterate and others are achieved a variety of educational levels. Nel *et al*. (2004) highlight the education of food handlers as a crucial line of defense in the preventions of most types of food-borne illnesses. Training about hygiene during handling and cooking of food items is very important.

4. 5. 2. Personal hygienic practices of food handler's

In the current study a total of 30 food handlers were assessed on personal hygienic practice using an observation check list. The results of personal hygienic practices of food handlers are shown in (Table 5).

Twelve (40%) of the food handlers do not wash their hands and drying before adding the milk in to the cup for fermentation, 26 (86.7) of the food handlers do not wear aprons (gowns), 19 (63.3) do not have hair covered, 27 (90) wore hand jewelries and 25 (83.3) of the food handlers do not keep finger nails short and avoid nail polished during food preparation (Table 4).

Table 5: personal hygienic practices of food handlers in Resturants and cafeterias of Bahir Dar town, Ethiopia, 2014

Practice	Frequency	Percent
Washing hands with soap and drying		
Yes	18	60
No	12	40
Wear apron, gown		
Yes	4	13.3
No	26	86.7
Hair covered		
Yes	11	36.7
No	19	63.3
Worn hand jewelry		
Yes	27	90
No	3	10
Keep finger nails short and Avoid nail polished		
Yes	5	16.7
No	25	83.3

Personal hygiene is important because human beings are the largest contamination sources of food (Marriot, 1985).

Hand washing is an essential component of infection control (Larson, 2003).To get rid of germs and dirt, it is important to wash hands properly and frequently with detergents and warm water. Hands that have long nails or jewelry, such as rings and bracelets, are more difficult to clean thoroughly and can collect small pieces of debris and bacteria that does not wash off easily. False fingernails and nail polish are not allowed (Mekonnen Haileselassie *et al*., 2012).

Food handlers with poor personal hygiene working in food service establishments could be potential sources of infection to pathogenic organisms (Gashaw Andargie *et al.,* 2008). A study in the USA indicated that improper food handling practices contribute to 97% of foodborne illness in food service establishments and at home and food safety training has been shown to have a positive impact on practices of handlers (Howes *et al*., 1996). Therefore, training and motivation should be provided to the food handlers working in these establishments (Mulugeta Kibret and Bayeh Abera, 2012). According to FAO (1990), food handlers should have the necessary knowledge and skills to enable them to handle food hygienically.

4. 5. 3. Sanitary conditions of utensils and the vending environment:

In the current study sanitary conditions of 30 homemade yoghurt selling restaurants and cafeterias were assessed using interview and an observation check list. The results of sanitary conditions of homemade yoghurt are shown in (Table 6).

One (3.3%) of the homemade yoghurt selling restaurant has private toilet and others 29 (96.7%) of the restaurants have no toilets, 12 (40%) have piped private water supply and others use shared piped water supplies, 16 (53.3%) properly covered the milk cups to make yoghurt and 14 (46.7%) the milk cups not properly covered, only 7 (23.3%) wash cups and covers with soap and drying and 23 (76.7%) not properly washing and drying utensils, 29 (96.7%) of raw milk source is purchased from outside source and only 1 (3.3%) from their own, 14 (46.7%) uses refrigerator to store yoghurt after fermentation

Table 6: The sanitation conditions of utensils and the vending environment in Bahir Dar town, Ethiopia, 2014.

Parameter		Frequency	Percent
Private toilet			
	Yes	1	3.3
	No	29	96.7
Private water piped supply			
	Yes	12	40
	No	18	60
Properly cover the milk cup			
	Yes	16	53.3
	No	14	46.7
Wash cups and covers with soap and drying before adding the raw milk			
	Yes	7	23.3
	No	23	76.7
Source of the raw milk			
	Their own	1	3.3
	Out side	29	96.7
Have domestic animals			
	Yes	2	6.7
	No	28	93.3
Source of yoghurt			
	on site	29	96.7
	premade	1	3.3
Storage of yoghurt			
	Refrigerator	14	46.7
	Room temperature	16	53.3
Storage and transportation material			
	Plastic	22	73.3
	Stainless	8	26.7
Product out come at the end of the day			
	Discard	5	16.7
	stored	25	83.3
Hygienic situation of area used for selling yoghurt			
	Clean	13	43.3
	Not clean	17	56.7

and 16 (53.3%) stay the yoghurt at room temperature until selling, 22 (73.3%)uses plastic materials to ferment and transport the milk and 5 (16.7%) end of the day and only 8 (26.7) uses stainless materials, 25 (83.3%)stored the product outcome at the end of the day and 5 (16.7%) discard the product at the end of the day, 17 (56.7%) of the area is contaminated by dust and smoke and 13 (43.3%) of the area is clean. Domestic animals were found in 6.7% of the homemade yoghurt selling restaurants.

Results from studies done in Mekelle on the Source (s) of contamination of 'raw' and 'ready-to-eat' foods and their public health risks revealed that 20% of the respondents did not have some food and utensils were not covered properly, which could result in food contamination due to dust and microbes. The utensils in which the food is displayed for sale must be kept clean, covered and protected as they easily become contaminated if left dirty or unprotected. Foodstuffs of all kinds should be kept covered as much as possible to prevent contamination from dust and flies (Mekonnen Haileselassie *et al*., 2012).

Food items should be stored and handled correctly to decrease the growth of the microorganisms already present and to minimize the risk of contamination (Gorman *et al*., 2002). However results from studies done in Mekelle on the Source(s) of contamination of 'raw' and 'ready-to-eat' foods and their public health risks revealed that 35.5% of the respondents did not have chilling facilities for perishable food (Mekonnen Haileselassie *et al*., 2012).

Studies conducted in Bahir Dar on food safety practices of the food handlers have revealed that 16% of the raw materials were stored at room temperature. More than 50% of the food handlers prepare food at the peak selling time where as about 50% of the left over is consumed by the food handlers themselves and their families or sold the following day (Mulugeta Kibret and Bayeh Abera, 2012).

Most ergo houses were centers at the main roads which would expose them to contamination by dust particles, equipment's and storage containers. Additional handling of ergo into different plastic containers and sieves may cause the contamination of ergo, since as the number of plastic containers and sieves increased the chance of contamination is also increased and most plastic containers have characteristics that make them unsuitable for milk handling (Shima *et al.,* 2012).

Food hygiene is vital throughout the whole food chain from raw materials to transportation of ready products. The main routs of contamination of ergo include insufficient pre-milking udder preparation, Insufficient cleaning of milker hands and milking utensils, use of poor quality and non-boiled water for cleaning of udder, transportation of milk and the way it is offered for sale. Food handlers should have a staff training program in place that provides all employees with the knowledge and skills they need to produce a safe product within the processing premises (Ukwo *et al.,* 2011).

5. CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

The bacteriological quality of all homemade yoghurt samples from restaurants and cafeterias of Bahir Dar town showed that the aerobic mesophilic count, the total coliform count and the fecal coliform count exceeded the acceptable limit recommended. This may cause human health risk due to consumption of those homemade yoghurt products. This high number should be due to lack of hygiene and sanitary problems. Hence, adequate sanitary measures should be taken at stage of from production to consumption. The detection of *Salmonella* in 9 (30%) homemade yoghurt samples regarded as potentially hazardous to consumers and is unacceptable for consumption. Poor hygienic practices by food handlers together with poor sanitary conditions in restaurants and cafeterias can contribute to outbreaks of food borne illnesses. Educational programs targeted to change the attitude of food handlers have been recommended. In addition, Periodic sanitary-hygienic evaluation and inspection should be conducted regularly.

5.2. RECOMMENDATIONS

Based on the findings and limitations of the present study, the following recommendations are therefore made:

- Further research must be conducted on enumeration of bacteria from food utensils, food handlers, bacteriological examination of the water used for the washing.
- Professional training on food handling should be launched to change the attitude and handling practice of service providers or vendors.
- Due to lack of facilities and time, there was a limitation of this study for doing antimicrobial susceptibility test and serological tests for the isolated pathogens. So it recommends for the future to do antimicrobial susceptibility test and serological tests for each pathogenic bacteria isolated for yoghurt samples. Due to lack of time the sample size of this study also minimum.

6. REFERENCES

Abd EI-Atty, S. N. and Meshref, S. M. A. (2007). Prevalence of *salmonella* and *E.coli* 0157 in some Foods. *Veternary Medicine Journal*. 5th Scientific conference. 73-78.

Abdelgadir, W. S., Ahmed, T. K. and Dirar, H. A. (1998). The traditional fermented milk products of Sudan. *International Journal of Food Microbiology.* **44**: 1-13.

Abebe Bereda, Mitiku Eshetu and Zelalem Yilma (2014). Microbial properties of Ethiopian dairy products. *African Journal of Microbiology Research*. **8**(23): 2264-2271.

Acha PN, Szyfers B (2001). Zoonoses and Communicable Diseases Common to Man and Animals: Bacteriosis and Mycosis. 3rded.Vol I. Washington DC: Pan American Health Organization. pp. 233-246.

Adams, M. and Motajemi, Y. (1999). Basic Food Safety for Health. World Health Organization. Geneva.

Adams, M. R. and Nout, M. J. R. (2001). Fermentation and food safety. In: *Australian Manual for control of salmonella in the Dairy Industry*, Pp. 1-45, (Australian Dairy Authorities Standards Committee (ADASC), eds). Springer, US.

ADASC Food Author (2000). Minimum Sampling Guide Lines for Dairy Products.

Ahmad, I., M. Gulzar, F. Shanzad, M. and Yaqub Zhoor. (2013). Quality Assessment of yoghurt produced at large (industrial) and small scale. *Journal of Animal and Plant Sciences.* **23**: 58-61.

AI- kadamany, E., Toufeili, I., Khattar, M., Abou-Jawdeh, Y., Haraleh, S. and Haddad, T. (2003). Determination of shelf life of concentrated yoghurt (Labneh) production bag straining of set yoghurt using analysis. *Journal of Dairy Sciences.* **85:** 1023-1030.

Akabanda, I. Owusu-Kwarteng, J. K., Gloveri, R.L. and Tano-Debrah, K. (2010). Microbiological characteristics of Ghanaian traditional fermented milk product, nunu. *Nature and Science*. **8**(9): 178-187.

Almaz Gonfa, Alemu Fite, Kelbessa Urga and Berhanu Abegaz Gashe (1999). Microbiological aspects of 'Ergo'('Ititu') fermentation. SINET: *Ethiopian Journal of Science*. **22:** 283-289.

Almaz Gonfa, Foster, H A. and Holzapfel, W. H. (2001). Field survey and literature review of Ethiopian traditional fermented milk products. *International Journal of Food Microbiology.* **68**: 173-186.

Aloys, N. and Angeline, N. (2009). Traditional fermented foods and beverages in Burundi. *Food Research International.* **42**: 588-594

Amakoromo, E. R., Innocent-Adiele, H. C. and Njoku, H. O. (2012). Microbiological quality of a yoghurt-like product from African yam bean. *Journal of Nature and Science*. **10:** 6-10.

Amha Kebede, Viljoen, B.C., Gadaga, T.H., Narvhus, J.A. and Lourens-Hattingh, A. (2007). The effect of container type on the growth of yeast and lactic acid bacteria during production of *Sethemi*, South African spontaneously fermented milk. *Food Research International*. **40:** 33-38.

Aneja, R. P., Mathur, N. B., Chandan, C. R. and Banerjee, K. A. (2002). Technology of Indian Milk products; A Dairy Indian publication, Delhi.

Asaminew Tassew and Eyassu Seifu (2011). Microbial quality of raw cow's milk collected from farmers and cooperatives in Bahir Dar Zuria and Mecha district. *Agriculture and Biology Journal of North America.* **2**(1): 29-33.

Atanda, O. O., and Ikenebomeh, M. J. (1991). Microbiological quality of *nono*. *World Journal of Microbiology and Biotechnol*ogy. **7**: 89-91.

Aycicek, H., Oguz, U. and Karci, K. (2006). Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. *Internet Journal* of *Hygiene* and *Environment.* **209**: 197–201.

Baumier, A. J., Tsolis, R. M. and Heffron, F. (2000). Virulence Mechanisms of *Salmonella* Their Genetic Basis. *Salmonella* in Domestic Animals. CAB International, Wallingford, UK.

Bille, P.G., Buys E. and Taylor, J.R.N. (2007). The technology and properties of *Omashikwa*, traditional fermented buttermilk produced by small-holder milk producers in Namibia. *International Journal of Food Science and Technology.* **42**: 620-624.

Boor, K. J., Brown, D. P., Murphy, s. c and Bandler, D. K. (1998). Microbial and chemical quality of raw milk in New York State, *Journal of Dairy Science.* **8**: 1743-1748.

Campel- platt, G. (1994). Fermented foods-A World perspective. *Food Research International*. **27**: 253-257.

Caplice, E. and Fitzgerald, G.F. (1999). Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology*. **50**: 131-149.

Centers for Disease Control (CDC). (2007). National Antimicrobial Resistance Monitoring system for Enteric Bacteria (NARMS): human isolate final report, 2007. CDC, US Department of Health and Human Services. Atlanta. GA.http://www.cdc.gov/narms/annual/2007/narmsannualreport2007.pdf. (Accessed on 7 February 2013).

Chambers, J.V. (2002). The microbiology of raw milk, In: Dairy microbiology Hand book, 3rd edition. New York: *Wiley-Inter science*. Pp. 39-89.

Cross, J. H., George, R. H., Booth, I. W. and Mayne, A. J. (1989). Life-threatening *Salmonella Enteritidis* Phage Type 4 Gastroenteritis in Infancy. *Lancet*. **868**: 625-626.

CSA (Central Statistical Authority of Ethiopia) (2011). The 2010 Population and Housing Census of Ethiopia.

D'aoust, J. Y. (1994). Salmonella and the international food trade. *International Journal of Food Microbiology.* **24**:11-31.

D'aoust, J. Y. (1995). Salmonella and the international food trade. *International Journal of Food Microbiology.* **24**:11-31.

David, G., Slack, R.C.B. and Peutherer, J.F (2006). Medical Microbiology: A Guide to Microbial Infections, Pathogenesis, Immunity, Laboratory Diagnosis and Control. 16th ed. Thomsom Press Ltd. Pp. 37-60.

Deriba Mulgeta and Mogessie Ashenafi (2001). *Salmonella*, *Shigella* and Growth potential of other foodborne pathogens in Ethiopian street vended foods. *East African Medical Journal.* **78**: 576-580.

Desalegn Amenu (2013). Antimicrobial activity of Lactic acid bacteria isolated from "Ergo" Ethiopian traditional fermented milk. *Current Research in Microbiology and Biotechnology*. **1**(6): 278-284.

Dogan, B. and Boor, K. J. (2003). Genetic diversity and spoilage potentials among Pseudomonas spp. Isolated from fluid milk products and dairy processing plants. *Applied Environmental Microbiology***. 69**: 130-138.

El-Gendy, S.M. (1983). Fermented Foods of Egypt and the Middle East. *Journal of Food Prot.* **46**: 358-367.

El-Malt LM, Abdel Hameed KG and Mohammed AS (2013). Microbiological evaluation of yoghurt products in Qena city. *Egypt Veterinary World.* **6**(7): 400-404.

Eleanor, S. (2007). A survey of the microbiological quality of sweet baked foods. Government of South Australia Department of Health. 1-7.

Environmental Fact Sheet (2003). Fecal Coliform as an Indicator Organism. New Hampshire. 271-350.

ENVR431 (2008). Indicator Bacteria-Total and Fecal coliforms, *E. coli*. Multiple Fermentation Tube (MFT) or "Most Probable Number" (MPN) Methods and Membrane Filter (MF) Methods. Techniques in Environmental Health Sciences.

FAO (1990). Street foods: Report of FAO expert consultation. Jogjakarta, Indonesia. *FAO Nutrition*. **46**: 3-30.

FAO (1997). Agriculture food and nutrition for Africa. A resource book for teachers of agriculture. Rome.

Farzana, K., Akhtar, S. and Jabeen, F. (2009). Prevalence and antibiotic resistance of bacteria in two ethnic milk based products. *Pakistan Journal of Bot*any. **4**: 935-943.

FEHD (2007). Microbiological Guidelines for Ready-to-eat food, Food and Environmental Hygien Department, Queensway, Hong Kong.

Fekadu Beyene (1994). Present situation and future aspects of milk production, milk handling and processing of dairy products in Southern Ethiopia. Food Production Strategies and limitations: The case of Aneno, Bulbula and Dongora in southern Ethiopia. PhD. Thesis. Department of Food Science Agriculture. University of Norway.

Feresu, S.B. and Muzondo, M. I. (1990). Identification of some lactic acid bacteria from two Zimbabwean fermented milk products. *World Journal of Microbiology and Biotechnol*ogy. **6**: 178-186.

Foltys, V. and Kirchnerova, K. (2006). Mesophilic and psychrotrophic aerobic sporulating microorganisms in raw milk. *Archiva Zootechnica.* **9**: 41-55.

FSAI (2001). Guidelines for the Interpretation of Results of Microbiological Analysis of some Ready-to eat Foods Sampled at point of sale, Food Safety Authority of Ireland, Dublin, Ireland. Guidance Note No.3. 1-12.

Gashaw Andargie, Afework Kassu, Feleke Moges, Moges Tiruneh and Kahsay Huruy. (2008). Prevalence of Bacteria and Intestinal Parasites among Food-handlers in Gondar Town, Northwest Ethiopia. *Journal of Health, Population and Nutrition*. **26**(4): 451–455.

Gilmour, D. (1999). Milking. **In**:*Small holder Dairy in Tropics.* (Falvey, L.,Chantalakhana,C.,)

ILRI, Nairobi Kenya PP.36-37.

Godefay, B. and Molla, B. (2000). Bacteriological quality of raw milk from four dairy farms and milk collection center in and around Addis Ababa.Berl.Munich. Tierarzil wschr.**113**, PP.1-3

Gudeta, M. (1987). Isolation and Identification of enteric bacteria in raw milk produced.

Hahn, G. (1996). Pathogenic bacteria in raw milk-Situation and significance. Symposium on bacteriological quality of raw milk, Wolfpassing, Austria. PP. 60-64.

Hamdan, R. H., Musa, N., Musa, N., Wei, S. L., and Sarman, A. (2008). Isolation and Enumeration of Coliform Bacteria and *Salmonella* spp. from Short Necked Clam *Orbicularia orbiculata* at East Coast, Malaysia. *Internet Journal of Food Safety.* **10:** 58-64.

Havlova , J., Jicinska, E. and Hrabova, H. (1993). Microbiological methods in quality control of milk and milk products. Praha: UZPI, ISBN. **80**: 120-137.

Hobbs, B.C. and Roberts, D. (1993). Food poisoning and food hygiene. 6th edition. London: Edward Arnold publishers. Pp. 3-373.

Howes M, McEwen S, Griffiths M, Harris L. (1996). Food handler cortication by home study: Measuring changes in knowledge and behavior. Dairy, Food Environ Sanitation. 16:737–744.

Huyam E. Hamad, Abdel Moneim E.Sulieman and Zakaria A. Salih. (2013). Quality Aspects of the Sudanese Fermented Milk (*Robe*) Supplemented with Gum Arabic powder. *Discourse Journal of Agriculture and Food Sciences.* **1**(1): 8-7.

ISO 4832 (BS 5763 Part 2) (1991). Microbiology-General Guidance for the enumeration of Coliforms-Colony count Technique. Geneva: International Organization for Standardization (ISO).

Jacob, R. (2010). Microbial Quality of Ready-to-Eat Foods Available to Populations of Different Demographics. Drexel University, Philadelphia.

Karagozlu, N. Karagozlu, C. and Ergonul, B. (2007). Survival Characteristics of *E.coli* O157:H7, *S. typhimurium* and *S. aureous* during Kefir fermentation. *Czech Journal of Food Science*. **25**(4): 202-207.

Kosikowski, F. V. (1997). Cheese and fermented milk foods, Volume one, ISBN, pp. 1058.

Kurmann, J.A., Rasic, J. Lj. and Kroger, M. (1992). Encyclopedia of Fermented Fresh Milk Products. An International Inventory of Fermented Milk, Cream, Buttermilk, Whey, and Related products. Van Nostrand Reinhold. ISBN 0-442-00869-4 New York. USA.

Kurwijila, R.L., Hansen, K.K.,Macha, I.E., Abdallah, K. and Kadigi, H.J.S.(1992).The bacteriological quality of milk from hand and machine milked dairy herds in Morongo, Tanzania, *Journal of African Livestock Research* **2**:59-67.

Larson, E., Aiello, A., Lee, LV., Della-Latta, P., Gomez-Duarte, C. and Lin, S. (2003). Short and long term effects of hand washing with antimicrobial or plain soap in the community. *Journal Community Health.* **28**(2): 139-50.

Lefoka, M. (2009). The survival of microbial pathogens in dairy products. Faculty of natural and agricultural sciences, Department of microbial, Biochemical and Food Biotechnology, University of the Free State, Bioemfontein.

Lewis, Joy E., Patrina Thompson, Rao BVVBN, Kalavati C, Rajanna B. 2006. Human bacteria in street vended fruit juices: A case study of Visakhapatnam city, India. *Internet Journal of Food Safety.* 8:35 -38. Linton, A. H. (1982). Microbes , Man and animals .The Natural History of Microbial Interaction, 1st edition . John Willey and Sons Ltd. PP. 150-151.

Liyuwork Tesfaw, Biruhalem Taye, Sefinew Alemu, Haile Alemayehu, Zufan Sisay and Haileleul Nigussie (2013). Prevalence and antimicrobial resistance profile of salmonella isolates from dairy products in Addis ababa, Ethiopia. *Global Journal of Industrial Microbiology*. **1**(1): 23-27.

Mankee, A., Ali, S., Chin, A., Indalsingh, R. Khan, R., Mohammed, F., Rahman, R., Sooknanan, S., Tota-Maharaj, R., Simeon, D. and Adesiyun, A. A. (2003). Bacteriological quality of

"doubles" sold by street vendors in Trinidad and the attitudes, knowledge and perceptions of the public about its consumption and health risk. *Food Microbiology*, 20: 631-639.

Marcos, A., Warnberg, J., Nova, E., Gomez, S., Alvarez, A., Alvarez, r., Mateos, J. A., Cobo, J. A. and Cobo, J. M. (2004). The effect of milk fermented by yoghurt cultures plus *Lactobacillus casei* DN-114001 on the immune response of subjects under academic stress. *Europian Journal of Nutrition*. **43**: 381-389.

Marriot, N., (1985) Principles of food sanitation. NewYork: van Nostrand Reinhold Company, 70-80.

Massoun, Z. and Nadine, N. (2010). Influence of production processes in quality of fermented milk "Laban" in Lebanon. *Journal of Medicine and Health* care. **2**(4): 381-389.

Motaremi,Y.and Nout R. J. M. (1996). Food fermentation: A safety and nutritional assessment. *Bulletin of the World Health Organization*. **74**(6): 553-559.

Mckinley, M.C. (2005). The nutrition and health benefits of yoghurt. Internet *Journal of Dairy Technology.* **58**: 1-2.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S. and Shapiro, C. (1999). Food-related illnesses and death in the united states. *Emerging Infectious Diseases*. **5**: 607-625.

Meerburg, B.G. and Kijlstra, A. (2007). Role of Rodents in Transmission of *Salmonella* and *Campylobacter*. *Journal of Science Food Agriculture.* **87**: 2774-2781.

Mekonnen Haileselassie, Habtamu Taddele and Kelali Adhana (2012). Source(s) of contamination of 'raw' and 'ready-to-eat' foods and their public health risks. *ISABB Journal of Food and Agriculture Science.* **2**(2): 20-29.

Mensah, P. (1997). Fermentation - the key to food safety assurance in Africa. *Food Control*. **8**: 271-278.

Ministry of Health (2004). Planning and Programming Department: Health and Health Related Indicators. Health Information Processing and Documentation Team.

http://cnhde.ei.columbia.edu/indicators/Health_Indicators96. *(accessed* December 20 , 2012).

Miskimin, D.K., Berkowitz, K.A., Solberg, M., Riha, J.R., Franke, W.C., B.Uchanan, R. L. and O'leary, v. (1976). Relationship between indicator organisms and specific pathogens in potentially hazardous foods. *Journal of Food Science*. **50**: 336-339.

Mogessie Ashenafi (1992). Growth potential and inhibition of *Bacillus cereus* and *Staphylococcus aureus* during the souring of "Ergo", a traditional Ethiopian fermented milk. *Ethiopian Journal of Health Development***. 6**: 23-30.

Mogessie Ashenafi (1995). Microbial development and some chemical changes during the making of 'Ergo', traditional Ethiopian fermented milk. *Bulletin Animal Health production Africa*. **43:**171-176.

Mogessie Ashenafi (2002). The microbiology of Ethiopian foods and beverages: A review.SENET: *Ethiopian Journal of Sci*ence. **25**: 97-140.

Mogessie Ashenafi (2006). A Review on the microbiology of indigenous fermented foods and beverages of Ethiopia: A review. *Ethiopian Journal Biological Science*. **5**: 189-245.

Mulugeta Kibret and Bayeh Abera (2012). The Sanitary Conditions of Food Service Establishments and Food Safety Knowledge and Practices of Food Handlers in Bahir Dar Town. *Ethiopian Journal of Health Sciences.* **22**: 27-35.

Mourad, K. and Nour-Eddine, K. (2006). Physicochemical and microbiological study of "shemn", a traditional butter made from camel milk in the sahara (Algeria): isolation and identification of lactic acid bacteria and yeasts. **57**: 2.

Mudgil, S., Aggarwal, D. and Ganguli, A. (2004). Microbiological Analysis of Street Vended Fresh Squeezed Carrot and Kinnowmandarin Juices in Patiala City. *Indian International Journal of Food Safety*. **3**: 1-3.

Murphy, S. C. and Boor, K. J. (2003). Basic Dairy Bacteriology, Microbiological Quality Defects in Fluid Milk Products: The evaluation of shelf life Cornell University, Ithaca, NY.

Napravnikova, E., Vorlova, l. and Malota, L. (2002). Changes in hygienic quality of Vacuum-packed pork during storage. *Acta Veterinary Brno*. **71**(12): 255-262.

Nel, S., Lues, JFR., Buys, EM. and Venter, P. (2004). The personal and general hygiene practices in the deboning room of a high throughput red meat abattoir. *Food Control.* 15: 571-578.

Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Giessen, J. v. d. and Kruse, H. (2010). Food-borne diseases-the challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology.* 139: 3-15.

O'Connor, C.B. (1994). Rural Dairy Technology. ILRI Training manual No.1. International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia. Pp. 133.

Ombui, J.N.,Arimi, S.M.,Mcdermott, J.J.,Mbugua,S.K.,Githua,A.A.,and Muthoni J.(1995).Quality of raw milk collected and marketed by dairy cooperative societies in kiambu District, Kenya Bulletin of Animal health prod. Africa **43**:277-285.

Oyewole, O.B. (1997). Lactic fermented foods in Africa and their benefits. *Food Control*. **8:** 289-297.

Patrica, M. and Azanza, V. (2004). Aerobic plate counts of Philippine ready to rat foods from take away premises. *Journal of Food Science and Nutrition.* **2**: 103-107.

Pazakova, j., Turek, p. and Laciakova, A. (1997). The survival of *Staphylococuus aureus* during the fermentation and storage of yoghurt. *Journal of Applied Microbiology*. **82**: 659-662.

Pethers, J.V.S. and Gilbert, R. J. (1971). Survival of *Salmonella* on finger tips and transfer of the organism to foods. *Journal of Hygiene.* **69**(4): 673-681.

Ray, B. (2004). Fundamental Food Microbiology. Third edition. CRC Press. Washington. D.C. Pp. 188-195.

Ray, B. and Bhunia, A. (2008). Fundamental Food Microbiology. 4th ed. CRC Press. Taylor and Francis Group. Boca Raton London. New York.

Reddy, B.U., Chandrakanth, Indu, P. S., Nagalakshmi, R. V. and Usha, B. K. (2009). Isolation and characterization of fecal coliforms in Street vended fruit juces and its safety evaluation: A Case study of Bellary city, India. *Internet Journal of Food Safety*. **11**: 35-43.

Richardson, G. H. (1985). Standard Methods for the Examination of Dairy Products. 15th ed. American public Health Association Washington, D. C. Pp. 48-94.

Rodrigues, L.A., Ortalani, M.B.T. and Nero, L.A. (2010). Microbiological quality of yoghurt commercialized in Vicosa, Minas Gerais, Brazil. *African Journal of Microbiological Research***. 4**: 210-213.

Savadogo, A., Ouattara, T. A. C., llboudo, J. A., KAROU, D. and Traore, S. A. (2010): Isolation and susceptibility to antibiotics of bacterial strains from Burkinafaso fermented milk samples. *Advanced Journal of Food Science and Technology*. **2**(2): 91-95.

Scherer, C.A. and Miller, S.I. (2001). Molecular pathogenesis of *Salmonellae.* In Groisman. E.A. (Ed). Principles of bacterial pathogenesis, 265-316. United States of America: Academic Press.

Skriver, A., Holstborg, J. and Qvist, K. B. (1999). Relation between sensory texture analysis and rheological properties of stirred yoghurt. *Journal of Dairy Research.* **66:** 609-618.

Smith, S. and Schaffner, D. W. (2004). Indicator Organisms in meat. Enc. Meat Sci. Elsevier Science, London. Pp.612.

Tekinsen K. K. and Ozdemir Z. (2006). Prevalence of food borne pathogens in Turkish Van otlu (Herb cheese. *Food Control*. **17**: 707-711.

U.S Department of Agriculture, (1981). USDA Fact Sheet Number 57. Pp. 4-5.

Valemynck.G.(1994).The Significance of Salmonella organisms in Raw milk , International Dairy Federation , Brussels, Belgium. PP.77-80.

Vasavanda, C.P. (1993). Rapid methods and automation in dairy microbiology. *Journal of Dairy Science.* **76:** 3101-3106.

Mensah, P. (1997). Fermentation - the key to food safety assurance in Africa. *Food Control.* **8**: 271-278.

Younus, S., Masud, T. and Aziz, T. (2002). Quality evaluation of market yoghurt /Dahi. Pakistan *Journal of Nutrition.* **1**: 226-230.

Zelalem Yilma, Bernard, F. and Gerard, L. (2007). Occurrence and distribution of species of Enterobacteriaceae in selected Ethiopian traditional dairy products: A contribution to epidemiology. *Journal of Food Control.* **18**:1397-1404.

Zelalem Yilma (2010). Microbial properties of Ethiopian marketed milk and milk products and associated critical points of contamination: An Epidemiological perspective, East Africa Dairy Development (EADD) Program, Addis Ababa Ethiopia.

APPENDIX

Table 1: Aerobic mesophilic count of yoghurt samples

Sample number	Cfu/ml	Log cfu/ml
1	6,300000	6.799340
2	56,400000	7.751279
3	45,700000	7.659916
4	43,600000	7.639486
5	37,100000	7569373
6	6,300000	6.799340
7	44,500000	7.648360
8	43,400000	7.637489
9	43,000000	7.633468
10	42,900000	7.632457
11	43,200000	7.635483
12	36,900000	7.567026
13	42,700000	7.630427
14	43,000000	7.633468
15	53,000000	7.724275
16	36,500000	7.562292
17	34,500000	7.537819
18	4,000000	6.602059
19	34,500000	7.537819
20	39,300000	7.594392
21	39,800000	7.599883
22	4,150000	6.618048
23	4,180000	6.621176
24	4,150000	6.618048
25	32,700000	7.514547
26	35,000000	7.544068
27	32,600000	7.513217
28	35,000000	7.549827
29	33,100000	7.507855
30	32,200000	7.507855

Table 2: Total coliform count of yoghurt samples

Sample Number	Cfu/ml	Log cfu/ml
1	3,900	3.591064
2	3,770	3.576341
3	3,810	3.580924
4	3,820	3.582063
5	3,650	3.562292
6	3,710	3.569373
7	3,730	3.571708
8	4,400	3.643452
9	439	2.642464
10	4,460	3.649334
11	3,720	3.570542
12	3,800	3.579783
13	3,820	3.582063
14	3,420	3.534026
15	3,720	3.570542
16	3,470	3.540329
17	407	2.609594
18	3,820	3.582063
19	403	2.605305
20	3,590	3.555094
21	3,620	3.558708
22	3,600	3.556302
23	3,590	3.555094
24	2,130	3.328379
25	2,390	3.378397
26	3,320	3.521138
27	3,390	3.530199
28	3,300	3.518513
29	3,730	3.571708
30	327	3.514547

Table 3: Fecal coliform count of yoghurt samples

Sample Number	Cfu/ml	Log cfu/ml
1	377	2.576341
2	3,730	3.571708
3	376	2.575187
4	375	2. 574031
5	3,650	3.562292
6	3,610	3.569373
7	3,630	3.559906
8	393	2.594392
9	396	2.597695
10	3,730	3.571708
11	398	2.599883
12	3,720	3.570542
13	382	3.582063
14	4,050	4.484299
15	3,080	4.488550
16	331	2.519827
17	335	2.525044
18	334	2.523746
19	291	2.463892
20	390	2.591064
21	387	2.587710
22	385	2.585460
23	389	2.589949
24	2,070	3.315970
25	2,150	3.332438
26	3,090	3.489958
27	3,140	3.496926
28	2,980	3.474216
29	366	2.563481
30	3,300	3.518513

Table 4: Biochemical tests for identification of salmonella

No	Isolate code	Citrate utilization	Sugar Indole motility (SIM)			Sugar(TSI) Fermentation	Lysine Utilizationgas and H_2S formation	Urease production
			H_2S Production	Motility	Indole			
1	Sa96	+	+	-	+	A/g	+	-
2	Sa911	-	+	-	+	A/g	+	-
3	Sa916	+	+	-	+	A/g	+	-
4	Sa918	+	+	-	+	A/g	+	-
5	Sa920	+	+	-	+	A/g	+	-
6	Sa925	+	+	-	+	A/g	+	-
7	Sa927	+	+	-	+	A/g	+	-
8	Sa928	-	+	-	+	A/g	+	-
9	Sa930	+	+	-	+	A/g	+	-

A: Acid, K:Alkaline, g: gas (+):positive, (-): negative

Table 5: Minimum Test Guide Lines for yoghurt samples

Bacteriological Test	**Microbiological quality (cfu/gm)**				**Source**
	Good	**Acceptable**	**Unsatisfactory**	**Potentially hazardous**	
Aerobic mesophilic count		Not exceeding 150,000 cfu/ml			Australian Dairy Authorities'Standards Committee (ADASC) (2001)
Total coliform	<100	Not exceeding 100 cfu/ml 100-10^4 cfu/ml	≥10^4	N/A	ADASC (2001) FSAI (2000)
E.coli	<20	Not exceeding 10 cfu/ml 20-≤100	≥100	N/A	ADASC (2001). FSAI (2000)
Salmonella	Not detected in 25 gm	----	-----	Detected in 25 gm	NSW Food Authority (2009), ADAS (2000),

N/A= Not applicable

Interviews and Observational Check list

I. Handling practices of vendors

1. Do food handlers wash hands with soap and water regularly before the raw milk added in to the cup and after? a) Yes

b) No

2. Do food handlers wear apron (gown)? a) Yes

b) No

3. Do food handlers covered hair? a) Yes

b) No

4. Do food handlers worn jewelry? a) Yes

b) No

5. Do food handlers keep finger nails short and avoid nail polished? a) Yes

b) No

II. Sanitation condition of utensils and the vending environment

1. Have you private toilet? a) Yes

b) No

2. Have you private water piped water supply? a) Yes

b) No

3. Do properly cover the milk cup after adding the raw milk for making yoghurt? a) Yes

b) No

4. Do wash cups and covers with soap and drying before adding the raw milk? a) Yes

a) No

5. The source of the raw milk? a) Their own

b) Out side

6. Have you your own domestic animals? a) Yes

b) No

7. Where is the source of yoghurt? a) Made on site

b) Bought in premade

8. Yoghurt stored in a) Refrigeration

b) Room temperature

9. Storage and transportation material of yoghurt a) plastic

b) Stainless

10. Product out come at the end of the day a) Discard

b) Stored for the next day

11. Hygienic situation of area used for selling yoghurt a) clean

a) Not clean