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Evaluation of the Chemical and Microbial Properties of Domestic Commercial Pasteurized Milk Available in Addis Ababa, Ethiopia

By

Anteneh Mebratu

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirement For the Degree of Master of Science in Food Science and Nutrition.

July, 2015

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DECLARATION

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

Anteneh Mebratu

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The thesis has been submitted with my approval as thesis advisor.

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List of abbreviations and acronyms

APC	Aerobic Plate Count
APHA	American Public Health Association
CC	Coliform Count
CFU	Colony Forming Units
CRD	Completely Randomized Design
DDE	Dairy Development Enterprise
EEC	European Economy Community
EHNRI	Ethiopian Health and Nutrition Research Institute
ES	Ethiopian Standard
HTST	High Temperature Short Time
IDF	International Dairy Federations
LTLT	Low Temperature Short Time
ML	Milliliter
MPN	Most Probable Number
QSAE	Quality and Standard Authority of Ethiopia
RPM	Revolutions Per Minute
SNF	Solids Not Fat
TBC	Total Bacterial Count
TS	Total Solids
UDSS	Urban Dairy Sector Study
UHT	Ultra High Temperature
UNICEF	United Nations Children's Fund
UNRRA	United Nations Relief and Rehabilitation Administration
USDA	United State Department of Agriculture
USFDA	United States Food and Drug Administration
WHO	World Health Organization

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Abstract

Over the past few years private dairy processing industries have flourished and as a result the volume of domestic commercial pasteurized milk supplied to the urban market particularly to the capital has increased. However the chemical composition and microbial quality of these products is poorly understood. This study was conducted to evaluate the chemical and microbial properties from ten different domestic commercial pasteurized milk brands available in Addis Ababa. The mean total bacterial and coliform count was 1.4 x 10^7cfu/ml and 5.1 x 10^2cfu/ml respectively. These values exceeded the maximum acceptable regulatory limits of the Ethiopian Standards which is 10^5cfu/ml for total bacteria and 10 cfu/ml for coliforms. While E.coli should not be detected in heat treated foods, 60% of the samples analyzed were positive for E.coli and their mean value was 1.9 x 10^1MPN per gram. The average values of major chemical compositions were found to be; 2.95 % fat, 2.75 % protein, 9.45 % total solids and 6.46 % solids not fat. The values of the chemical compositions, except for the fat, were below the minimum regulatory limit of the Ethiopian Standards which requires 1.5 - 3.5 % fat, 3.2 % protein, 12.8 % total solids and 9.3 - 11.3 % solids not fat. The disparity between the results observed and the nutritional label was even higher. The poor microbial results suggest the possibility of inadequate plant cleanliness, low microbial quality raw material milk, defective pasteurization process and/or post pasteurization contamination. The likely causes for the lower values of chemical composition are the use of low quality starting milk or addition of water. Therefore this study could be helpful to processing industries, government regulatory bodies and consumers by providing information on the chemical and microbial qualities and suggestions for improvement opportunities.

Keywords: Pasteurized milk, Chemical quality, Microbial quality, Coliforms, E. coli

1. Introduction

Ethiopia holds large potential for dairy development mainly due to its large cattle breed diversity and population; the favorable climate for improved, high-yielding dairy cattle breeds; and the relatively disease-free environment for livestock. Milk and milk products form part of the diet for many Ethiopians, who consume dairy products either as fresh milk or after processing into diverse products (Ahmed *et al.,* 2004).

Dairying is practiced almost all over Ethiopia involving a vast number of small, medium and large-sized; and subsistence and market oriented farms. Based on climate, landholdings and integration with crop production as criterion, different dairy production system can be identified in Ethiopia that: include pastoralists, agro-pastoralists, mixed crop-livestock; peri-urban and urban (Sintayehu *et al*., 2008).

Total milk production in Ethiopia increased during the 1961-2000 period at an average annual rate of 1.55% though per capita production declined as a result of the high population growth rate. However, during the last decade production grew at a higher rate of 3% (SNV, 2008). The dairy sector in Ethiopia is expected to continue growing over the next decades given the large potential for dairy development practices in the country, the expected growth in income, increased urbanization, and improved policy environment (Ahmed *et al.,* 2004).

Though it constitutes a small proportion of the total milk produced, the emergence of private dairy processing industries significantly increased the volume of domestic pasteurized milk in the market. This resulted in an estimated urban sale of processed milk at 150,000 l/day (Land O'Lakes, 2010).

As milk products play an important role in human nutrition throughout the world, and as consumers are increasingly becoming concerned about quality, products must be not only acceptable but also of high quality to protect consumer health from food borne diseases. In less developed areas and especially in hot tropics high quality of safe product is most important, which is not an easy objective to achieve though (De Graaf *et al*., 1997).

Milk is a complex biological fluid and rich in food nutrients thus a good growth medium for many spoilage as well as pathogenic microorganisms. Milk obtained form a healthy cow is almost sterile as it comes from the udder. Microbial contamination therefore occurs post milking due mainly to mishandling; and once entered into milk, microorganisms multiply rapidly rendering milk unfit for further processing and/or unsafe consumption. The microbial content of milk is a major feature in determining its quality (Fernandes, 2009). Milk and milk products can, therefore, represent a health risk to consumers if contaminated by any pathogens and kept at high temperatures that favor microbial multiplication to high counts, which then may produce toxins (Radostitis *et al*., 1994; O'Mahony, 1988).

While pasteurization destroys many microorganisms and improves the keeping quality of milk; poor initial milk quality, defective pasteurization process, improper handling after pasteurization and problems in preservation at the retail outlets may result into microbial contamination in milk and thus great chances of deterioration of quality of milk (UDSS, 2006)

Since it is widely consumed by all age groups of the population all over the world, milk has been marked as one of the most important foods for which chemical and microbial quality control is a requisite (Saha and Ara, 2012). Though the safety of dairy products with respect to food-borne diseases is of great concern around the world, it is more important especially in developing countries where the required dairy infrastructure including cold chain is less developed (Yilma and Faye, 2006). In countries where food borne illness are investigated and documented, the relative importance of pathogens such as *Staphylococcus aureus, E.coli, Salmonella* species and *Listeria* species is well known (Godefay and Molla, 2000).

2. Statement of the problem

The world still faces with substantial problems related to food borne diseases associated with contamination of the food supply. The WHO estimates that each year there are 1.3 million cases of active diarrhea in children less than five years in the developing world. A substantial number of these cases are due to microbial contaminated foods. As reported by Ehlers (1976) Tuberculosis, typhoid fever, dysentery, diphtheria, septic sore throat and other streptococcal infections, staphylococcal toxins, salmonella gastroenteritis, and brucellosis are among the diseases transmitted via milk; while reports on the microbiological quality and safety of milk and milk products from Ethiopia are scanty (Ashenafi, 1992). It should also be noted that much of foods are lost as a result of spoilage caused, among others, by spoilage organisms (WHO, 1996).

Over the past decade several small- and medium-scale commercial dairy processing enterprises have been established and entered into the market in addition to the few existing ones. Their principal produce is pasteurized liquid milk packed in 500ml plastic bags. Indeed, production and consumption of pasteurized milk has increased especially in the capital, Addis Ababa and major cities and towns in its environ. With the economy growing and emergence of middle class the demand for pasteurized milk is expected to grow even further.

Quality evaluation of processed dairy products, in terms of their microbial and chemical attributes, conducted in several countries revealed that the products and most importantly commercial pasteurized milk are not always meeting applicable quality standards (Saha and Ara, 2012; Petrus *et al.,* 2010; Okpalugo, *et al.,* 2008). In Ethiopia, the limited earlier researches have also revealed the seriousness of the problem. In his study on the microbial quality evaluation from six brands of domestic commercial pasteurized milk marketed in Addis Ababa; Yilma (2012), for instance, reported that the mean bacterial, coliform and enterobacteriacea counts were much higher than acceptable values.

Given the lack of strong and functional regulatory enforcement on quality control in the country and the limited availability of research reports; this study was conducted to bridge the information gap on the chemical and microbial qualities of domestic pasteurized milk marketed in Addis Ababa.

3. Objectives of the study

3.1. General objective

To examine the chemical and microbial qualities of selected domestic commercial pasteurized milk products distributed in Addis Ababa that will contribute to the improvement of the commercial dairy sector through providing information and suggesting interventions.

3.2. Specific objectives

- To determine the contents of quality indicator organisms; total bacterial count, coliform count and e. coli in domestic commercial pasteurized milk products distributed in Addis Ababa
- To determine the major chemical compositions: fat, protein, total solids and solids not fat in domestic commercial pasteurized milk products distributed in Addis Ababa

3.3. Significance of the study

- Provides useful information for regulatory inspection bodies whether marketed pasteurized milk meet minimum acceptable and legal standards
- Increases knowledge and understanding of producers and consumers on sanitary and chemical qualities as well as safety of commercial pasteurized milk
- Gives feedback to dairy processing industries on the microbial and chemical qualities of their products and helps them ensure delivering safe and quality pasteurized milk to consumers
- Provides information on the prevailing situation pertaining to the chemical and microbial qualities of domestic pasteurized milks distributed in Addis Ababa, which can serve as a baseline evidence allowing improvement interventions by the concerned stake holders

4. Literature review

4.1. Overview of dairy development in Ethiopia

In the first half of the 20th century, dairying in Ethiopia was mostly traditional. The first attempt to introduce modern dairy production in the country was started in the early 1950s when 300 Friesian and Brown Swiss dairy cattle were received as a donation from the United Nations Relief and Rehabilitation Administration (UNRAA). With the introduction of these cattle in the country, commercial liquid milk production started on large farms in Addis Ababa and Asmara (Ahmed *et al.,* 2004; SNV, 2008).

To facilitate growth of the sector, UNICEF established a public sector pilot processing plant at the Sholla area, whose location, in 1960, was at the outskirt of Addis Ababa. The plant started by processing milk produced by large state owned dairy farms. The plant significantly expanded in a short period, established collection centers and started collecting milk from smallholder producers within a radius of about 150km from Addis Ababa in all directions. This led to further expansion of large dairy farms (Ahmed *et al.,* 2004).

Following a change in the economic policy during the mid-1970s, the government shifted attention from urban producers to rural producers. However, substantial resources remained devoted to establishing large-scale state farms to provide liquid milk for urban consumers. As a result the Dairy Development Enterprise (DDE) was established by merging the milk processing plant with numerous other nationalized dairy farms. The enterprise had a daily processing capacity of 60,000 liters of milk at its inception (SNV, 2008).

After the introduction of the market oriented economic policy in the beginning of the 1990s, the first private dairy processing plant was established in Sebeta town (25km south west of Addis Ababa) in 1998 (UDSS, 2006). The private sector constitutes an important part of the dairy sector. It is engaged in providing farm inputs (feed and veterinary drugs), animal health care and milk processing and storage equipment and serves as an important market outlet for milk and milk products (Yilma *et al.*, 2011).

Over the last 10 years, the number of commercial milk processing plants increased significantly raising processing capacity and dairy product lines. Currently, there are over 20 registered medium- and large-scale dairy processing companies in Ethiopia with about half of them operating in and around Addis Ababa and the rest in other major regional cities. Their capacities range from less than 1,000 liters per day to 60,000 liters per day. This resulted in an estimated milk intake by these major processors of about 150,000 liters per day (Yilma *et al.*, 2011; Land O'Lakes, 2010).

Table 1: Major private dairy enterprises operating in different parts of Ethiopia

Ser. No.	Dairy enterprise	Location	Year of establishment	Daily processing capacity, (litres)	Attained average capacity, (litres)
1	Sebeta Agro Industry (Mama Dairy)	Sebeta	1998	35 000	30 000
2	Lame Dairy Processing (former DDE)	Addis Ababa	2008	60 000	30 000
3	Dire Dawa Dairy Processing Enterprise	Dire Dawa	1972	20 000	20 000
4	MB PLC (Family Milk)	Addis Ababa	2003	15 000	7 000
5	Yadeni Dairy Farm (Bora Milk)	Addis Ababa	2008	15 000	7 000
6	Ada'a Dairy Cooperative	Debre Zeit	1998	15 000	3 000
7	Lema Dairy	Debre Zeit	2004	10 000	3 000
8	Berta and Family plc	Addis Ababa	2000	9 000	6 000
9	Genesis Farm	Debre Zeit	2001	4 000	4 000
10	Holland Dairy	Debre Zeit		4 000	4 000
11	Almi Tiku Wetet (Almi Fresh Milk)	Hawassa		4 000	3 000
12	Ruth and Hirut Dairy Farm	Addis Ababa	2008	4 000	1 500
13	Abay fana Awash Agro-Industry	Adama		3 500	2 000
14	Chuye Milk and Milk Products Processing	Addis baba		3 000	1 000
15	Fantu and Family Dairy Farm	Addis Ababa		2 500	2 000
16	Zemen Milk	Mekelle		2 000	150
17	Pinguin International Business plc (cheese world)	Addis Ababa		1 800	600
18	Life Milk Processing Enterprise	Sululta		1 500	1 500
19	Semit Agro Industry/Enat Milk	Mojjo			
20	Beral Milk	Addis Ababa	1991		
21	Harmonius Agro Industry	Adama			
22	Jantekel Dairy Union (Facil Milk)	Gonder		1 200	300

Source: (Yilma *et al.*, 2011; Land O'Lakes, 2010)

4.2. Chemical and nutritional composition of milk

Milk contains nearly all the nutrients necessary to sustain life such as proteins, lipids, lactose, minerals, vitamins, enzymes, etc. The biological function of milk is to supply nutrition and immunological protection to the young mammal. In some species, milk is the only food consumed for weeks or months. Nutritionally, milk has been defined as "the most near perfect food". The nutritional value of milk as a whole is greater than the value of its individual nutrients because of its unique nutritional balance (O'Mahony, 1988; Ralph, 1998).

Table 2: Composition of cow's milk (typical figures)

<table>
<tr><th>Component</th><th>% w/w</th><th>Total Solids (TS)</th><th>Solids-not-fat (SNF)</th></tr>
<tr><td>Water</td><td>86.5</td><td colspan="2"></td></tr>
<tr><td>Fat</td><td>4.5</td><td rowspan="4">13.5</td><td></td></tr>
<tr><td>Protein</td><td>3.5</td><td rowspan="3">9.0</td></tr>
<tr><td>Lactose monohydrate</td><td>4.8</td></tr>
<tr><td>Minerals</td><td>0.7</td></tr>
</table>

Source: (Chemical analysis in the New Zealand Dairy Industry; Hughes and Gray, 2006)

Cow milk typically contains about 3.5 to 5% fat, composed mainly of triglycerides (95 – 96%), which is dispersed throughout the milk in globules. In addition to providing milk's characteristic taste and texture, fat supplies vitamins A, D, E and K, as well as certain essential fatty acids (Clarence *et al*., 1982). The most important protein in milk is casein; accounting for 80% of milk protein. Other proteins present in milk include albumin and globulin. Lactose (milk sugar) is the principal carbohydrate found in milk that makes up about 5% of the milk. In addition, milk is an excellent source of various minerals, mainly calcium and phosphorous, and also vitamins A and B2 (Jensen, 1995).

The composition of milk varies considerably with the breed of cow, stage of lactation, feed, season of the year, and many other factors. However, some relationships between constituents are very stable and can be used to indicate whether any tampering with the milk composition has occurred (Ralph, 1998).

Measures of milk quality evaluation for chemical composition include determination of the contents of fat, proteins, lactose, total solids and solids-not-fat (SNF). Total solids (TS) represent the components that remain after the complete removal of water from milk whereas solids-not-fat (SNF) is the difference between the total solids content and that of fat. Determination of acid in milk is also an important factor in judging milk quality because acidity percentage is a measure of freshness and bacterial activity in milk (Belitz *et al.,* 2009; Saha and Ara, 2012).

4.3. Pasteurization

Pasteurization is the process of heating milk to such temperatures, well below its boiling point, and for such periods of time as are required to destroy any pathogens which may be present, whilst causing minimal changes in the composition, flavor and nutritive value. The thermal destruction process is logarithmic, and bacteria are killed at a rate that is proportional to the number of bacteria present (Jeffrey and Paivi, 2009; O'Mahony, 1988). The process was named after Louis Pasteur who discovered that spoilage organisms could be inactivated in wine by applying heat at temperatures below its boiling points. The process was later applied to milk and remains the most important operation in the processing of milk (Namminga, 1999).

Pasteurized milk presents little health hazard as it has the advantage of reducing the transmission of disease producing bacteria, decreasing infant mortality, and improving the keeping quality of milk (Clarence, 1990). Nevertheless, several food-borne diseases out breaks have been linked to pasteurized milk and traced to inadequate pasteurization, post pasteurization contamination or storage temperature abuse. Microbial spoilage of pasteurized milk is also of concern, since milk supports abundant growth of microorganisms, leading to development of off-flavors, coagulation and ropiness (ICMSF, 1998).

Since food borne diseases represent one of the most widespread and overwhelming public health problems of the modern world, bacteriological analysis of milk should be made frequently on all ingredients and daily on the mix and frozen products (FAO/WHO, 1970; WHO, 2000). The increasing number of outbreaks associated to dairy products has highlighted the importance of microbiological control in the dairy industry (Vasavada and Cousin, 1993).

Though, numerous time/temperature combinations are recommended for pasteurization, the most commonly used one is heating milk at 72°C for 15 seconds followed by rapid cooling to below 10°C. This is normally referred to as High Temperature Short Time (HTST) treatment. It is carried out as a continuous process using a plate heat-exchanger to heat the milk and a holding section to ensure that the milk is completely pasteurized. In the case of Low Temperature Long

Time (LTLT) treatment fixed quantities of milk are heated to 63°C and held at this temperature for 30 minutes and then cooled at 5°C before packing. This is referred to as batch pasteurization where milk quantities are too small to justify the use of a plate heat-exchanger (O'Mahony, 1988; Belitz *et al.,* 2009).

4.3.1. Effect of pasteurization

Pasteurization kills many fermentative organisms as well as pathogens. Microorganisms that survive pasteurization are putrefactive. Although pasteurized milk has storage stability of certain days, subsequent deterioration is cause by putrefactive organisms. Thus, pasteurized milk will putrefy rather than developing acidity (O'Mahony, 1988).

The process has little effect on the nutritive value of milk. The major nutrients are not altered. There is some loss of C and B group vitamins, but this is insignificant. Though pasteurization does not reduce the fat content of milk it reduces the cream layer, since some of the fat globule membrane constituents are denatured. This inhibits clustering of the fat globules and consequently reduces the extent of creaming (Tetra Pak, 1995).

4.3.2. Methods/Types of pasteurization

4.3.2.1. Batch pasteurization

In the batch pasteurization system milk is heated or cooled in individual batches in one, two or sometimes three tanks. It is the oldest method of pasteurization that uses a vat pasteurizer which consists of a jacketed vat surrounded by either circulating water, steam or heating coils of water or steam. In the vat the milk is heated and held throughout the holding period while being agitated. The milk may be cooled in the vat or removed hot after the holding time is completed for every particle (Namminga, 1999).

4.3.2.2. Continuous pasteurization

In the continuous system, where a high temperature short time (HTST) pasteurizer is used, the filling, holding and discharging operations are carried out automatically in a timed cycle. The heat treatment is accomplished using a plate heat exchanger that consists of a stack of corrugated stainless steel plates clamped together in a frame. Milk is forced to flow between the metal plates or through pipes heated on the outside by hot water (Smith, 1981).

4.3.2.3. Ultra high temperature pasteurization

Ultra High Temperature (UHT) pasteurization is a modern HTST system where milk is treated in a continuous flow at higher temperatures and held for much shorter periods than in batch and continuous systems. Another method of attaining UHT is through aseptic processing which involves heating the milk using commercially sterile equipment and filling it under aseptic conditions into hermetically sealed packaging. The product is termed "shelf stable" and does not need refrigeration until opened (Namminga, 1999; Belitz *et al.,* 2009).

4.4. Microbial quality of pasteurized milk

Proper pasteurization checks out most if not all vegetative mesophilic spoilage and pathogenic microorganisms. This should, however, be followed by appropriate packaging and storage. Otherwise, pasteurized milk can be prone to contamination. Causes of contamination of pasteurized milk and types of contaminants are briefly discussed below.

4.4.1. Causes of spoilage of pasteurized milk

Improperly packed and stored pasteurized milk provides a very suitable environment for microbial growth and is therefore highly susceptible to microbiological spoilage. Spoilage may result from either the growth of psychrotrophic thermoduric organisms that survive pasteurization, or post-pasteurization contamination by psychrotrophs. The latter is considered to be by far the most common cause of spoilage (Fernandes, 2009).

4.4.1.1. Thermoduric spoilage

The thermoduric microflora of milk consists largely of Gram-positive spore formers, mainly *Bacillus* spp., *Clostridium* and organisms with heat-resistant vegetative cells, such as *Micrococcus*, *Lactobacillus*, *Enterococcus*, *Streptococcus*, *Corynebacterium* and *Alcaligenes*. Of these, the spore-formers are most important in spoilage, since the other species are not generally psychrotrophic and are unable to grow in refrigerated milk (Fernandes, 2009).

4.4.1.2. Post-pasteurisation contamination

The majority of post-process contaminants are Gram-negative bacteria, which may have some resistance to sanitizers and be able to colonize milk contact surfaces downstream of the pasteurizer. Initially, *Enterobacteriaceae*, such as *Enterobacter, Cronobacter,* and *Citrobacter*, predominate, but Gram-negative psychrotrophs, principally pseudomonas, but also *Alcaligenes*, *Klebsiella*, *Acinetobacter* and *Flavobacterium*, are more important in terms of eventual spoilage (Fernandes, 2009; Marth and Steele, 2001).

In the case of Ultra High Temperature (UHT) processed products spoilage is caused by post-process contamination that usually occurs as a result of a failure in the integrity of the aseptic filling system, or, more likely, as a result of packaging defects, such as pinholes or faulty seals. The product may then become contaminated with a variety of organisms of environmental origin and the type of spoilage is dependent on the nature of the contaminant (Marth and Steele, 2001).

4.4.2. Pathogens associated with milk with particular emphasis to pasteurized milk

There are several disease causing microorganisms that are associated with milk and milk products (Table 3). Some of the major ones particularly those associated with pasteurized milk are briefly discussed below:

Salmonella: Many strains of *salmonella* can cause food borne illnesses in humans, and all strains exhibit similar symptoms such as gastroenteritis (vomiting and diarrhea) (Jay, 2000). *Salmonella* is not able to survive the typical minimum pasteurization processes generally prescribed in legislation. Therefore, its presence in pasteurized products indicates that the process has not been carried out effectively, or that post-process contamination has occurred (USDA, 1981).

Listeria monocytogens: This widespread organism is found principally in soil. Listeriosis in humans may cause serious illness and is especially dangerous to pregnant women, causing stillbirths or infant death soon after birth (Pelczar *et al.,* 1965). *L. monocytogenes* is likely to be present in wet dairy processing environments, and post-process contamination is therefore a particular hazard. The organism has been shown to be capable of significantly more rapid growth in pasteurized milk than in raw milk at 7°C, and is also capable of multiplying at 4°C in pasteurized milk (USDA, 1994).

Campylobacter jejuni: This organism, isolated in raw milk and meat, can cause mastitis in dairy cattle. Symptoms include vomiting, cramps, bloody diarrhea, mild enteritis, or severe enterocolitis (Jay, 2000; USDA, 1981). *Campylobacter* spp. are not capable of surviving milk pasteurization treatments, nonetheless outbreaks of campylobacteriosis associated with pasteurized milk have been reported (Fernandes, 2009).

***Staphylococcus aureus*:** is a common cause of mastitis in dairy cattle and can enter the milk supply from sores on the teats of cows or from the hands and nasal discharges of dairy farmers and workers. The organism produces an enterotoxin (toxins causing vomiting and diarrhea) in raw milk when it is held at temperatures above 50 degree Fahrenheit. Sufficient amounts of enterotoxin in foods can cause illness (Jay, 2000). *S. aureus* is only rarely involved in food poisoning associated with consumption of pasteurized milk. This may be because it does not generally grow at temperatures below 7°C, and enterotoxin production is inhibited at low temperatures (USDA, 1994).

Bacillus spp.: psychrotrophic *Bacillus* spp. present in raw milk may survive pasteurization and then become dominant in the pasteurized milk, potentially causing spoilage. Concerns have been expressed that some psychrotrophic strains of *B. cereus* may be able to produce toxin in milk at refrigeration temperatures, but it seems likely that obvious spoilage would occur before sufficient toxin production had taken place to cause illness (Jay, 2000). Even so, *B. cereus* was isolated at levels of $4x10^5$ cfu/ml from pasteurized milk associated with 280 food poisoning cases in the Netherlands in 1989 (USDA, 1994).

Table 3: Human Microbial Pathogens Associated with Milk and Milk Products

Organism	**Disease**
Enterobacteriaceae	
Escherichia coli, including O157:H7	Gastroenteritis, hemolytic uremic syndrome
Salmonella	Gastroenteritis, typhoid fever
Yersinia enterocolitica (psychrotrophic)	Gastroenteritis
Other gram-negative bacteria	
Aeromonas hydrophila (psychrotrophic)	Gastroenteritis
Brucella spp.	Brucellosis (Bang's disease)
Campylobacter jejuni	Gastroenteritis
Pseudomonas aeruginosa	Gastroenteritis
Gram-positive spore formers	
Bacillus cereus (some strains are psychrotrophic)	Gastroenteritis
Bacillus anthracis	Anthrax
Clostridium perfringens	Gastroenteritis
Clostridium botulinum (type E is psychrotrophic)	Botulism
Gram-positive cocci	
Staphylococcus aureus	Emetic intoxication
Streptococcus agalactiae	Sore throat
Streptococcus pyogenes	Scarlet fever/sore throat
Streptococcus zooepidemicus	Pharyngitis, nephritic sequelae
Miscellaneous gram-positive bacteria	
Corynebacterium spp	Diphtheria
Listeria monocytogenes (psychrotrophic)	Listeriosis
Mycobacterium bovis	Tuberculosis
Mycobacterium tuberculosis	Tuberculosis
Mycobacterium paratuberculosis	Johne's disease (ruminants)
Rickettsia	
Coxiella burnetii	Q fever
Viruses	
Enterovirus, including polioviruses, rotaviruses, Coxsackie viruses	Enteric infection
FMD virus	Foot-and-mouth disease
Hepatitis virus	Infectious hepatitis
Fungi	
Molds	Mycotoxicoses
Protozoa	
Entamoeba histolytica	Amebiasis
Giardia lamblia	Giardiasis
Toxoplasma gondii	Toxoplasmosis

Source: Adapted from Boor, 1997, and Johnson et al., 1990: in Marth and Steele (2001)

4.4.3. Microbial quality indicators of pasteurized milk

Due to various reasons it is practically difficult to isolate all pathogens from pasteurized milk samples. Selected microbial tests, that are indicative of the general bacteriological quality, are routinely conducted to evaluate the microbial quality of pasteurized milk. These tests are determination of total bacterial count, coliform count and *E.coli* (Kiiyukia, 2003).

The total bacterial count refers to all viable microorganisms that could grow aerobically and form countable colonies on plate count agar incubated at 35^{o}C for 48hrs (Jay, 2000; Pelczar *et al.,* 1965). The result gives quantitative idea about the microbial load present in the sample in order to evaluate against guideline values. In legal matters concerning acceptability of incoming milk, the total bacterial count is the standard to which other screening tests are compared (Walstra *et al.,* 2005). The total bacterial count is a measure of hygienic quality of pasteurized milk. Excessively high counts are indicative of poor hygiene and may lead to early spoilage of pasteurized milk that makes it unfit for human consumption.

Coliforms are a group of bacteria that comprise all aerobic and facultative anaerobic, gram-negative, non-spore-forming rods able to ferment lactose and produce acid and gas at 35^{o}C within 48 hrs (Jay, 2000; Pelczar *et al.,* 1965). Coliforms can be found in the aquatic environment, in soil and on vegetation but they are commonly present in large numbers in the feces of warm blooded animals. While coliforms themselves are not normally causes of serious illness their presence is used to indicate that other pathogenic organisms of fecal origin may be present (Griffiths, 2000). *E. coli* is a rod-shaped member of the coliform group which is distinguished from most other coliforms by its ability to ferment lactose at 44°C. Unlike the general coliform group, *E. coli* is almost exclusively of fecal origin and its presence is thus an effective confirmation of fecal contamination. Most strains of *E. coli* are harmless, except for serotype O157:H7, which can cause food poisoning in but some can cause serious illness in humans and can become life threatening (Fernandes, 2009). Coliforms, faecal coliforms, and *E. coli* are destroyed by pasteurization and considered as indicator organisms. These microorganisms can also survive and multiply in a variety of non-intestinal environments, including the processing plant. Therefore their presence in heat treated milk is indicative of a defective pasteurization process or post pasteurization contamination (Kiiyukia, 2003).

4.5. Standards of pasteurized milk

Different organizations have been working in different countries to establish quality standards to ensure the health of consumers. Health hazards to the consumer are often grouped into microbiological, physical and chemical (Yilma *et al.*, 2011).

The United States FDA standards for Grade "A" pasteurized milk is indicated in Table 4 below.

Table 4: Chemical, Physical, Bacteriological, and Temperature Standards

GRADE "A" PASTEURIZED MILK AND MILK PRODUCTS	Temperature.....	Cooled to 7ºC (45ºF) or less and maintained thereat. **NOTE:** Milk sample submitted for testing cooled and maintained at 0ºC (32ºF) to 4.4ºC (40ºF), where sample temperature is >4.4ºC (40ºF), but ≤7.0ºC (45ºF) and less than three (3) hours after collection has not increased in temperature.
	Bacterial Limits**…..	Not to exceed 20,000 per mL, or gm.*** **NOTE:** Tested in conjunction with the drug residue/inhibitory substance test.
	Coliform.....	Not to exceed 10 per mL. Provided, that in the case of bulk milk transport tank shipments, shall not exceed 100 per mL. **NOTE:** Tested in conjunction with the drug residue/inhibitory substance test.
	Phosphatase****.....	Less than 350 milliunits/L for fluid products and other milk products by approved electronic phosphatase procedures.
	Drugs**.....….	No positive results on drug residue detection methods as referenced in Section 6 -Laboratory Techniques which have been found to be acceptable for use with pasteurized milk and milk products.

(Source: USFDA, 2011)

** Not applicable to acidified or cultured products, eggnog and flavored (non-chocolate) milk and milk products.

*** Results of the analysis of dairy products which are weighed in order to be analyzed will be reported in # per gm. (Refer to the current edition of the *SMEDP*.)

**** Not applicable to bulk shipped heat-treated milk products.

The microbial safety limits for pasteurized milk, UHT and sterilized milk as suggested by EEC Council Directives 92/46 (1992) are presented in Table 5.

Table 5: Microbiological safety limits for selected milk products in community legislation in force by the European Commission

Milk product	**Microorganism**	**Maximum limit (cfu/ml or g)**
Pasteurized drinking milk	Pathogenic microorganisms	Absent in 25g
	Coliforms	5
	Total bacteria	5×10^5
UHT milk and sterilized milk	Total bacteria	100

(Source: Yilma, 2012. in: Council Directives 92/46 EEC (1992)

Quality standards in Ethiopia have been published and issued by the Quality and Standards Authority of Ethiopia (QSAE), now called Ethiopian Standards Authority, which is the national standards body. The current collection of Ethiopian Standards (ESs) is mostly adopted from international standards. The implementation of or compliance with ESs is normally voluntary, however, standards that have direct influence on health, safety, etc are made compulsory (Yilma *et al.*, 2011).

Ethiopian Standards (requirements) for pasteurized liquid milk is indicated in Table 6 below.

Table 6: Ethiopian standards (requirements) for a pasteurized liquid milk

Requirements

Characteristics	**Requirements**	**Method of test**
Fat content, whole milk, min, % by mass	3.5	ES ISO 1211, ES ISO 2442
Fat content, Fat reduced milk, % by mass	1.5-3.5	ES ISO 1211, ES ISO 2442
Fat content, low fat milk, % by mass	0.5-1.5	ES ISO 1211, ES ISO 2442
Protein, min, % by mass	3.20	ES ISO 5542, ES ISO 8968-5, ES ISO 8968-1
Total solids, min, % by mass	12.80	ES ISO 6731
Phosphatase test	Negative	ES ISO 3356
Antibiotics	None	ES3473
Pesticide residues	See 13557	ES ISO 3890-1, ES ISO 3890-2
Salmonella	Nil	ES ISO 6785
Freezing point	0.525-0.550	ES ISO 5764

Microbial limits

Microorganisms/groups of microorganisms	**Requirement**
Total plate count	
Very good quality	<50000 per ml
Good quality	50000 – 100000 per ml
Fecal coliforms	Nil per ml
Non fecal coliforms	<10 per ml

(Source: Yilma *et al.*, 2011)

5. Materials and method

5.1. Study location

Collection of the samples was conducted in Addis Ababa while laboratory analysis was carried out at the dairy laboratory of the Ethiopian Meat and Dairy Industry Development Institute in Debrezeit, located 45 km south-east of Addis Ababa between February 2015 and May 2015. Addis Ababa is the capital city of Ethiopia located at 9°1′48″N latitude and 38°44′24″E longitude with altitude of 2450 meter above sea level. The mean annual rainfall is 1180mm and the mean minimum and maximum temperature is 10.6^{o}C and 22.8^{o}C respectively.

5.2. Study design

Commercially packaged (in 500ml plastic bags) domestic pasteurized milk samples were collected from different retail stores in Addis Ababa through random sampling method. The samples were kept in refrigerated box during collection and subsequent transport to the laboratory and were analyzed with in 6 hrs after collection (APHA, 1992). The pasteurized milk samples were between 3 to 5 days before expiry, as indicated on the packaging materials, at the time of collection and analysis. Ten different brands were considered for the present study. 5 units were randomly sampled from each brand at a time (USFDA, 2011). Triplicate tests were conducted for each brand. A total of 30 samples from ten brands were analyzed. For ethical considerations the samples were coded throughout the study.

5.3. Experimental procedure

5.3.1. Sample preparation

After withdrawing from the ice box, each commercially packaged pasteurized milk sample was shaken thoroughly for proper mixing. The outer surface of the packets was properly disinfected with 70% alcohol by using cotton cloth. The sample packets were opened with the help of sterile scissors. Each composite sample was prepared by mixing 50ml of the pasteurized milk transferred from the 5 sample units to make 250ml quantity. The required analytical sample was then taken from the composite sample.

5.3.2. Media and equipment preparation

Dehydrated commercial microbiological media were prepared according to the manufacturer's instruction. Distilled water was used to reconstitute and sterilization was carried out by means of steam sterilization in an autoclave at 121^{o}C for 15 minutes. All glass wares such as petri-dishes, pipettes, bottles, test tubes etc used for microbiological analysis were properly washed, dried and sterilized by means of dry heat sterilization in a hot air oven at 160^{o}C for 2 hrs. Analytical balance was calibrated with standard weight according to a written procedure prior to making measurements.

5.3.3. Microbiological analysis

Enumeration of total bacteria

After shaking the bottle containing the composite sample, 10ml of analytical sample was transferred into 90ml of sterile peptone water (PW) by means of a pipette and content was mixed together to make the first 1/10 dilution. Serial dilutions of 1/100, 1/1000, 1/10000, 1/100000 and 1/1000000 were prepared by progressive transferring of 1ml of the lower dilution in to 9 ml of peptone water to yield the next higher dilution. Each dilution was mixed thoroughly by a vortex mixer before the 1ml is withdrawn for serial dilution and a separate sterile pipette was used for making each transfer. Dilution levels of 1/100000 and 1/100000 were selected for culturing where each dilution was plated in duplicates. After agitation of each dilution bottle, 1 ml of the diluted solution was poured onto a petridish on which 12-15 ml of molten sterile Standard Plate Agar (SPC) was added and mixed thoroughly. When the solution in the petridish solidified it was put in the incubator at 35^{o}C in the inverted position for 48 ± 3hrs after which the number of bacterial colonies grown was counted. When the number of colonies was found too many, compromising the accuracy of counting, the same procedure was repeated using higher dilution levels (APHA 1992; Van den Berg, 1988).

The Total Bacterial Count (TBC) was computed from duplicate plates containing between 25-250 colonies. Plates containing less than 25 estimated counts and plates containing greater than 250 colonies for all dilutions were recorded as too few and too numerous to count respectively. For analysis purpose only counts in the normal range (25-250) were taken directly (APHA,

1992). To avoid fictitious impression of precision and accuracy, only the first two significant digits were reported by rounding up or down to the next number.
The following formula was used to calculate the counts:

$$N = \frac{\sum C}{[(1\ x\ n1) + (0.1\ x\ n2)]d}$$

Where

N:	Number of colonies per ml or g of product
∑C:	Sum of all colonies on all plates counted
n1:	Number of plates in first dilution counted
n2:	Number of plates in second dilution counted
d:	Dilution from which the first counts were obtained

Enumeration of coliforms

A 1 in 10 dilution of the milk was prepared by aseptically blending 10 ml of milk sample into 90 ml of peptone water (PW) in the same way as above. Serial dilutions of 1/100 and 1/1000 were prepared by progressive transferring of 1ml of the lower dilution in to 9 ml of peptone water to yield the next higher dilution. Dilution levels of 1/100 and 1/1000 were selected for culturing. Each dilution bottle was cultured in duplicates. After agitation of each dilution bottle, 1 ml of the diluted solution was poured on a petridish on which 12-15 ml of molten sterile Violet Red Bile Agar (VRB) was added and mixed thoroughly. When the solution in the petridish solidified it was put in the incubator at 35^{o}C in the inverted position for 48 ± 3hrs after which the number of bacterial colonies grown was counted. When the number of colonies was found too many, compromising the accuracy of counting, the same procedure was repeated using higher dilution levels (APHA, 1992; Marth and Stele, 2001).

The Total Coliforms Count (TBC) was computed from duplicate plates containing between 25-250 colonies in the same as described above for computing the Total Bacterial Count. The same formula was also used and final result was reported as cfu/ml (colony forming units per milliliter of sample.)

Detection and enumeration of E.coli

A 1 in 10 dilution of the milk was prepared by aseptically blending 10 ml of the milk sample into 90 ml of peptone water. Required serial dilutions were prepared. The samples were then inoculated into Lauryl Sulfate Tryptose (LST) broth containing inverted fermentation vials as follows: 10 ml of a 1/10 dilution into each of 3 tubes of double strength LST, 1 ml of 1/10 dilution into each of 3 tubes of single strength LST, and 1 ml of 1/100 dilution into each of 3 tubes of single strength LST. The inoculated LST broth tubes were incubated at 35^{o}C for 24±3 hrs. Positive LST broth tubes (gas production) were then inoculated to 10 ml Escherichia coli (EC) broth tubes containing inverted fermentation vials and were incubated at 44^{o}C for 24 ± 2 hrs. Production of gas constituted a positive E.coli test. Number of E.coli per ml of milk was computed from the most probable number table as indicated in the appendix (Kiiyukia, 2003).

5.3.4. Chemical analysis

Determination of total protein content

Formaldehyde titration method was used to determine the total protein content. 10 ml of milk was added into a beaker. Then 0.5 ml of 0.5% phenolphthalein indicator and 0.4 ml of 0.4 % Potassium Oxalate was added into the milk. The sample was then titrated with 0.1N Sodium Hydroxide solution. The titration was continued until pink color becomes intense. Finally, the burette reading was recorded. The reading was multiplied by a factor 1.74 (Foley *et al.,* 1974).

Percent protein = Burette reading x 1.74

Determination of total Solids content

Measurement of total solids content was carried out by oven drying method. Five grams of milk sample was placed in to a previously cleaned, dried and pre-weighed pan (porcelain crucible) in duplicate. Moisture removal was carried out in a two-stage process. Firstly, it was pre-dried over a steam bath before drying in an oven and then was placed inside a drying oven at 105^{o}C for

3hrs. After removed from the oven the pans were placed in to desiccators for 15 min. Finally the weight of the pans after drying was measured (O'Connor, 1994).

Total solids content of the milk was calculated as:

$$\%\text{Total Solids}\left(\frac{\text{wt}}{\text{wt}}\right) = \frac{\text{wt. of dry sample}}{\text{wt. of wet sample}} x100$$

The average of the duplicate values was recorded to determine the final content of total solids.

Determination of milk fat content

Gerber method was used to determine the milk fat content. Milk samples were kept at 37^{O}C for 30 minutes in a water bath to maintain the milk to normal body temperature of the cow. Ten milliliter of concentrated sulphuric acid was pipetted into a butyrometer (Gerber bottle). Then 11 ml of milk was added using milk pipette into a butyrometer having the sulphuric acid and then one milliliter of amyl alcohol was added. The butyrometer stopper was put on and the sample was shaken and inverted several times until all the milk was digested by the acid. Then the butyrometer was placed in a water bath at 65^{O}C for five minutes. The sample was placed in a Gerber centrifuge for four minutes at 1100 rpm (rotations per minute). Finally, the sample was placed in to water bath for 5 minutes at 65^{O}C and fat percentage was read from the butyrometer (ILCA, 1988; Van den Berg, 1988). The average of duplicate readings was computed and recorded.

Determination of solids not fat content

The solids not fat (SNF %) was determined by subtracting the percent fat from total solids (O'Mahony, 1988).

%SNF = (TS - fat) x 100

Where

SNF: Solids not fat

TS: Total solids

6. Results and Discussion

Results of the microbial counts as well as gross chemical composition of domestic pasteurized milk samples distributed in Addis Ababa are highlighted below.

6.1. Microbial quality

The mean total bacterial count (TBC) of the pasteurized milk samples of the current study ranged from 2.3 x 10^6 to 5.1 x 10^7 cfu/ml, the average value being 1.4 x 10^7, (as illustrated in Table 7). The U.S. Food and Drug Administration (FDA) guidelines detailed in the Pasteurized Milk Ordinance (PMO) require that following pasteurization, total bacterial numbers are not to exceed 20,000 cfu/ml (USFDA, 2011). The microbiological safety limit for pasteurized milk products in terms of total bacteria by the European Commission (EEC Council Directives 92/46, 1992) is 5 x 10^5 cfu/ml. The corresponding maximum total bacterial limit of pasteurized milk set by the Ethiopian Standards is 10^5 cfu/ml. The results are much higher than recommended by the FDA, EEC and ES and hence none of the pasteurized milk samples complied with the maximum allowed limit for total bacterial count.

Milk produced under hygienic conditions from healthy cows should not contain more than 5 × 10^4 bacteria per milliliter (O' Connor, 1994). Nonetheless the TBC of raw milk in Ethiopia was reported to be very high in several earlier studies; Godefay and Molla (2000) (1.9 × 10^8 cfu/ml), Mehari (1988) (10^7 - 10^9 cfu/ml), Hailu (1989) (1.7 × 10^7 - 7.5 × 10^7 cfu/ml), Tola *et al.* (2007) (7.4 x 10^7), Tolossa *et al.* (2012) (4.3 x 10^7). This suggests that the supply of raw milk quality to the processing plants does not meet acceptable standards. Excessively high bacterial counts can overwhelm the bacterial thermal destruction capacity of a pasteurizer, resulting in pasteurized milk with high bacterial numbers that may be unsafe to consume and that may have reduced quality and shelf life. High bacterial counts in raw milk can also suggest the presence of bacterially produced enzymes that may adversely affect the quality of any fluid milk and processed product made from the raw milk (Marth and Steele, 2001). The other reason for high bacterial count in the pasteurized milks might be due to lactic acid bacteria that can be explained by the low mean pH of 6.01 (Table 8); a pH value below 6.6 indicates acidity increases of milk

due to lactic acid bacterial multiplication (O'Connor, 1994). Microorganisms could survive pasteurization temperature if there is high level of contamination of raw milk and therefore keeping such pasteurized milk at room temperature for several hours in retail shops or households may lead to early spoilage of milk (Ashenafi and Beyene, 1994). Anderson and Stone (1955) also remarked that pasteurized milk might be contaminated due to the poor bacteriological quality of raw milk and inadequate plant cleanliness.

Table 7: Microbial quality of domestic commercial pasteurized milk marketed in Addis Ababa

Pasteurized milk sample*	Count, cfu/ml		MPN per gram
	TBC	TCC	E.coli
A	3.3×10^6	1.5×10^1	<3.0
B	5.1×10^7	1.1×10^2	3.2
C	7.7×10^6	2.0×10^1	<3.0
D	4.1×10^6	3.8×10^2	1.7×10^1
E	2.3×10^6	1.5×10^2	<3.0
F	2.3×10^7	1.3×10^3	4.8×10^1
G	4.3×10^6	2.2×10^1	<3.0
H	1.4×10^7	1.9×10^3	2.5×10^1
I	1.8×10^7	2.0×10^2	7.2
J	1.3×10^7	1.0×10^3	1.2×10^1
Over all mean	1.4×10^7	5.1×10^2	1.9×10^1

*Different letters represent different brands of pasteurized milk marketed in Addis Ababa;
TBC: Total Bacterial Count; TCC: Total Coliform Count; cfu/ml: Colony Forming Units per milliliter; MPN: Most Probable Number

Coliforms are considered as 'indicator organisms' because their presence in food indicates some form of contamination. The presence of coliforms and other gram negative bacteria in pasteurized milk can also be associated with unsanitary production and processing practices. Therefore they are good indicator of poor hygienic conditions during milk handling and processing. As it was observed in the present study the mean total coliform count (TCC) ranged from 1.5×10^1 to 1.9×10^3 with an average value 5.1×10^2 cfu/ml (Table 7). The presence of large numbers of coliform in foods is highly undesirable. The coliform organisms for pasteurized liquid milk should not exceed 5 cfu/ml according to the EEC directive where as the maximum safety limit is 10 cfu/ml as stipulated in both the US Food and Drug Administration and

Ethiopian Standard (ES). All the domestic pasteurized liquid milk samples tested in the current study had coliform counts much higher than the threshold limit. A positive test for coliform organisms in pasteurized milk greater than 10cfu/ml is an indication of improper processing or excessive contamination following pasteurization by improperly cleaned and disinfected equipment, utensils, or dipping of condensate into pasteurized milk (Salvato, 1992). Higher coliform counts in the raw milk supply chain were also reported; Dan *et al.* (2008) (4.6×10^2 to 8.7×10^5 cfu\ml), Tolossa *et al.* (2012) (7.5×10^6), Godefay and Molla (2000) (7×10^4cfu/ml) which may contribute to the elevated counts in pasteurized liquid milk.

Table 8: Mean pH of domestic pasteurized milk marketed in Addis Ababa

Pasteurized milk sample*	A	B	C	D	E	F	G	H	I	J	Overall Mean
pH	6.08	6.02	6.12	6.17	6.22	5.96	6.14	5.63	5.77	5.95	6.01

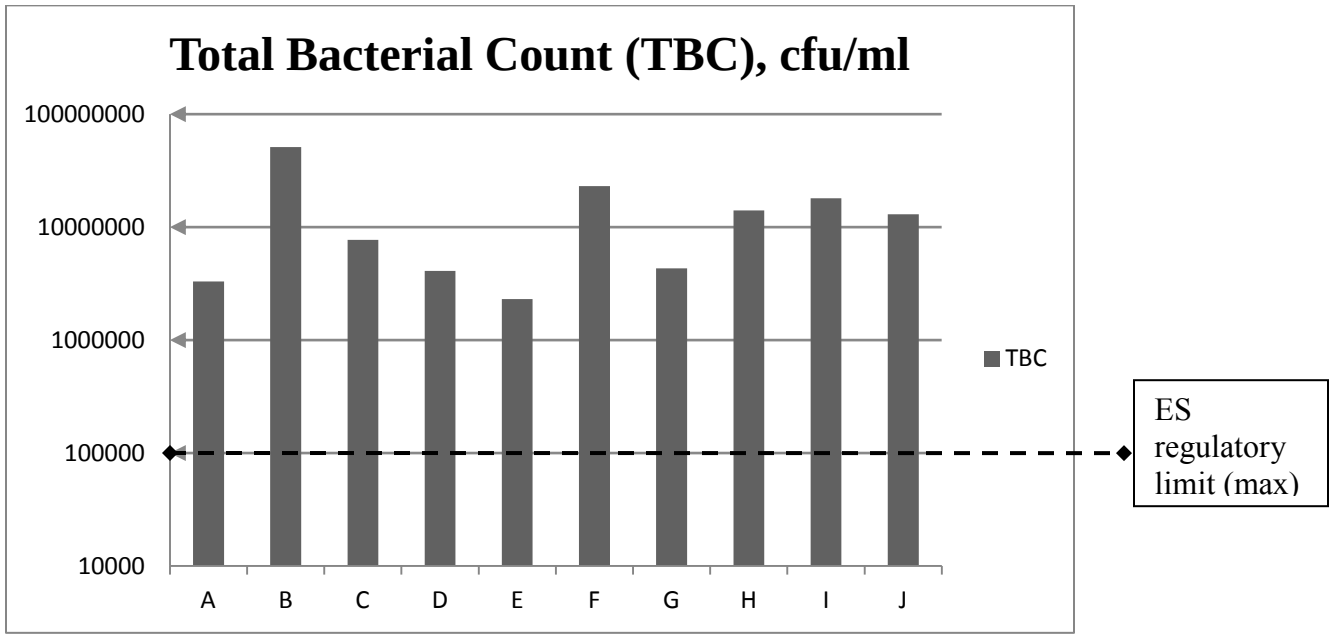
*Different letters represent different brands of pasteurized milk marketed in Addis Ababa

The results of the microbial quality obtained from the current study is only slightly lower than other similar study by Yilma (2012) where the mean TBC and TCC of domestic pasteurized milk samples from 6 brands marketed in Addis Ababa was 1.9×10^7 cfu/ml and TCC was 7.4×10^2 respectively. However, the results disagree with the finding of Asefa (2010) who found the mean TBC and TCC of pasteurized milk as 1.2×10^4 to5.2×10^5 cfu/ml and 0 cfu/ml to 2.27×10^4 cfu/ml respectively. The study was conducted from five processing plants located in the outskirts of Addis Ababa where pasteurized milk samples were collected from the pasteurizer unit itself before being packed in an effort to evaluate the operational efficiency of the pasteurizers. This suggests that the high bacterial and coliform count in pasteurized milk in retail packs might likely be associated to a possible post pasteurization contamination.

When compared to similar studies conducted in other countries, the result is still very much higher. For instance in Bangladesh Karim and Dey (2013) reported the microbial quality of pasteurized milk as 2.7×10^4 cfu/ml for TBC and 1.2×10^2 cfu/ml for TCC while a TBC of 6.1×10^4 cfu/ml and TCC of 1.3×10^1 cfu/ml was also reported (Sahah and Ara, 2012). Petrus *et al.* (2010) also reported the mean total bacteria count of 1.0×10^4 in pasteurized milk marketed in

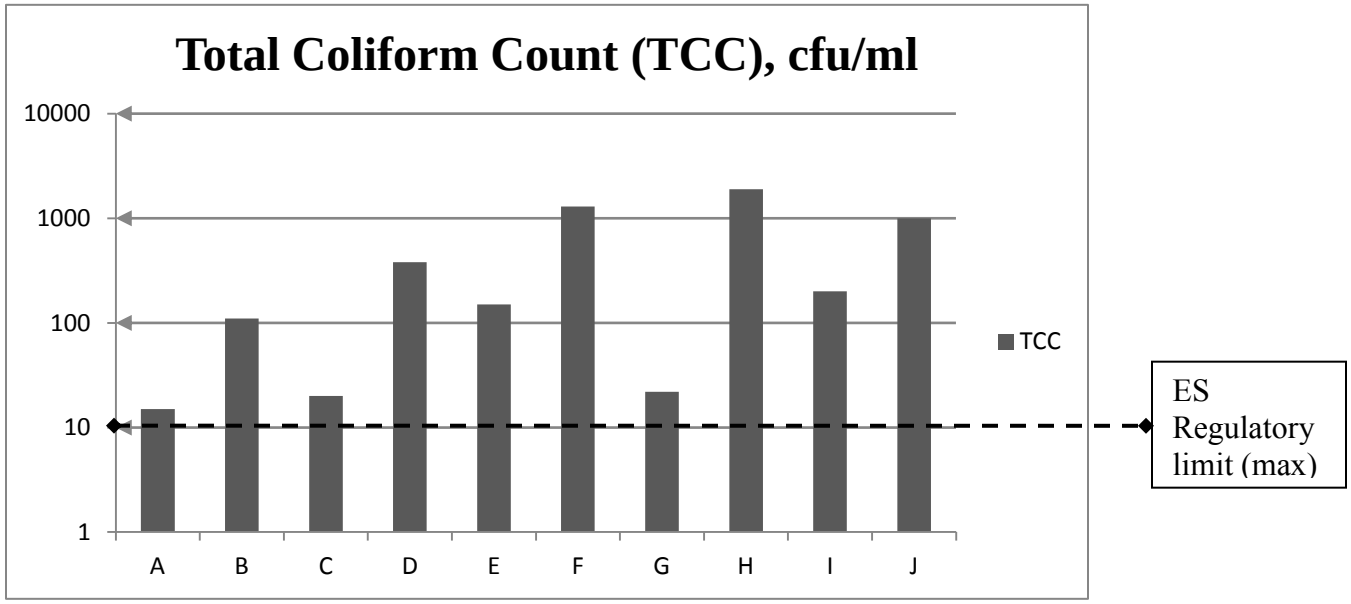
Brazil and similarly Hongfei *et al.* (2010) reported a total bacterial population of 2.0 x 10^4 cfu/ml for grade 'A" pasteurized milk available in Hawai'i, USA.

Figure 1: Comparison of total bacterial count of pasteurized milk marketed in Addis Ababa against regulatory limit of Ethiopian Standard (ES)



*Different letters represent different brands of pasteurized milk marketed in Addis Ababa

Figure 2: Comparison of total coliform count of pasteurized milk marketed in Addis Ababa against regulatory limit of Ethiopian Standard (ES)



The mean *E.coli* count in the present study ranged from <3.0 to 5.2 x 10^1 MPN per gram, the average value being 1.9 x 10^1 MPN per gram (Table 7). *E.coli* should not be detected in pasteurized milk moreover its presence in heat treated foods is usually associated with the presence of other pathogenic organisms. In the present study *E.coli* was not detected in only 4 out of 10 commercial pasteurized milk samples. That means *E.coli* was detected in 60% of the samples studied. Similarly Asefa (2010) reported the presence of *E.coli* organism in pasteurized milk in 40% of the samples investigated. But the result, which ranged from 0 cfu/ml to 2.2x10^4 cfu/ml, was much higher than that of the present study. Silva *et al.,* (2010) also reported a higher proportion (57.5%) of *E.coli* detection in samples of pasteurized milk tested in Brazil. A 15% detection of *E.coli* was reported by Salman and Hagar (2013) from two pasteurizing plants where pasteurized milk samples were collected at the point of sale in Khartoum, Sudan. Gogoy and Kaloianov (1978) reported fewer percentage of E.coli detection which was 11.76% in a pool of 212 pasteurized milk samples marketed in Bulgaria.

The detection of *E.coli* in the samples of pasteurized milk observed in the present study might suggest that there was a problem either in the pasteurization process or a post-pasteurization contamination due to poor processing and handling conditions and/or poor hygienic practices by the concerned employees. Post pasteurization contamination provides a serious obstacle to maintaining and extending fluid milk product shelf life. Two major sources contribute to post pasteurization contamination: equipment milk residues and aerosols. Ineffective cleaning procedures of the interior of processing equipment create milk residues which can allow bacteria to multiply and contaminate subsequent milk flow. Filler nozzles, carton-forming mandrels, and pasteurizers have all been pinpointed as sources of post pasteurization contamination (Gruetzmacher and Bradley, 1999; Ralyea et al., 1998).

6.2. Chemical quality

The average value of the mean fat content observed for the ten samples of domestic pasteurized milk was 2.95% (Table 9). The Ethiopian Standard requirement for fat content of pasteurized liquid milk (fat reduced milk) is between 1.5 and 3.5 %, in which range fall the values observed in the current study. The highest level of fat obtained was 3.43% and the lowest level was 2.42%. The value of fat content written in the packaging materials of the sample pasteurized milk is between 2.7 to 3.0 %. Accordingly nine out of the ten samples meet their own claimed content. Though the mean values of the fat content observed in the present study comply with the recommended standard, the results are much lower than the values of the raw whole milk as reported by several studies conducted in different parts of the country. Gemechu *et al.,* (2015) reported a mean fat content of 4.28% in raw milk collected from Shashemene in southern Ethiopia which is lower than that reported by Negash *et al,* (2012) 5.48% in mid rift valley area and Dehinenet *et al.,* (2013) 5.22 % from selected areas in Amhara and Oromia regions. A very high fat value of 6.05% was reported by Tola *et al.,* (2007) for raw cow milk studied from eastern wollega region in western Ethiopia.

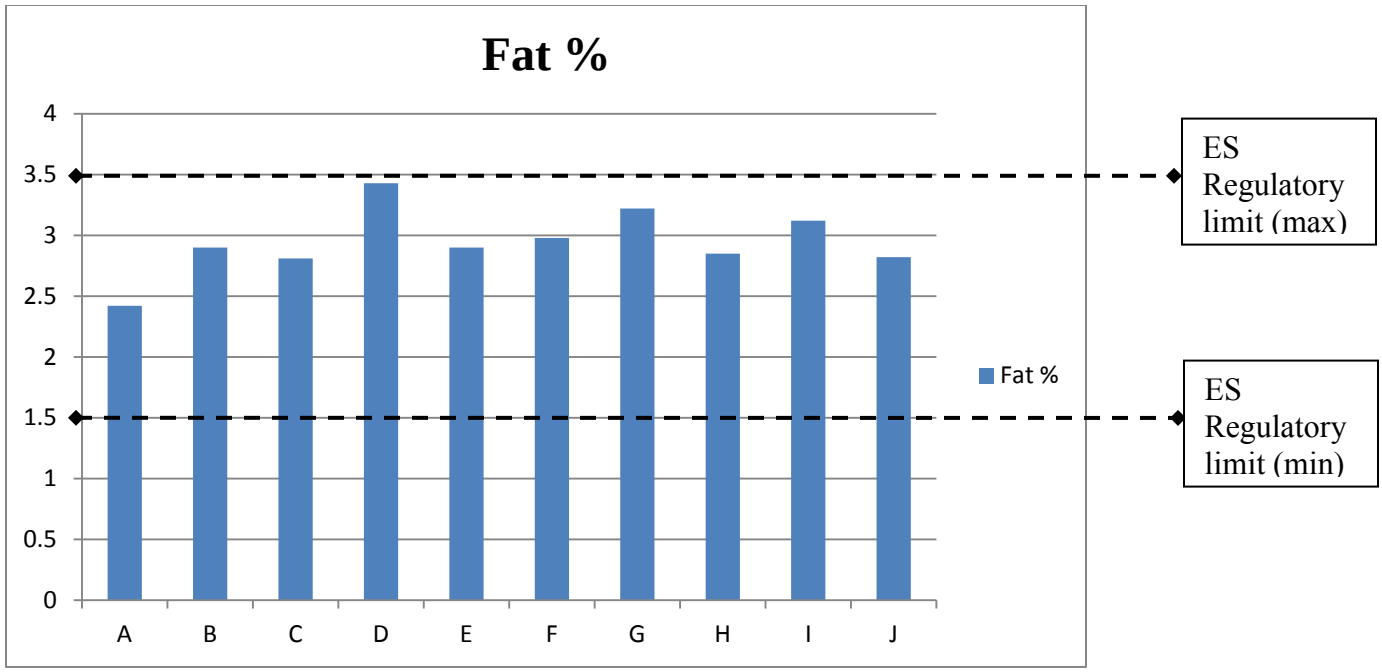
Table 9: Chemical composition of domestic commercial pasteurized milk marketed in Addis Ababa.

Pasteurized milk sample*	Fat %	Protein %	TS%	SNF %
A	2.42	2.34	7.87	5.45
B	2.90	2.72	9.21	6.31
C	2.81	2.61	8.82	6.01
D	3.43	2.96	10.38	6.95
E	2.90	2.80	9.45	6.56
F	2.98	2.80	9.50	6.53
G	3.22	2.94	10.00	6.91
H	2.85	2.75	10.13	6.65
I	3.12	2.80	9.64	6.52
J	2.82	2.73	9.51	6.70
Overall mean	2.95	2.75	9.45	6.46

*Different letters represent different brands of pasteurized milk marketed in Addis Ababa;
TS: Total Solids; SNF: Solids Not Fat

The reduction of fat level in the pasteurized milk may be the result of starting milk with a lower fat level than normal or may also be caused by the withdrawal of fat from the original milk that were used for pasteurization(Santos and Fonseca, 2000).

Figure 3: Comparison of fat content of commercial pasteurized milk marketed in Addis Ababa against regulatory limit of Ethiopian Standard (ES)



*Different letters represent different brands of pasteurized milk marketed in Addis Ababa

The highest and lowest values of total solids (TS) of pasteurized milk samples were observed to be 10.38%and 7.87%, respectively, with the overall average value being 9.45% (Table 9). The minimum Ethiopian Standard for pasteurized liquid milk with regard to total solids is 12.8% while the standard for solids not fat (SNF) content is between 9.3 to 11.3%. The mean total solids and solids not fat contents that refer to the total amount of materials dispersed in the aqueous phase, in all of the ten pasteurized liquid milk samples were much below compared with the recommended value and did not conform to the Ethiopian Standard. Even the highest contents of total solids (10.38%) and solids not fat (6.95%) values observed were by far less than the minimum requirement. However earlier studies on the contents of TS and SNF in raw whole

milk showed higher values when compared with the present study. A TS value of 12.87% (Gemechu *et al.,* 2015), 14.58% (Negash *et al.,* 2012), 13.66% (Dehinenet et al., 2013) and 14.31% (Tola *et al.,* 2007) was reported while the corresponding values for the SNF were 8.59%, 9.10%, 8.44% and 8.22% respectively.

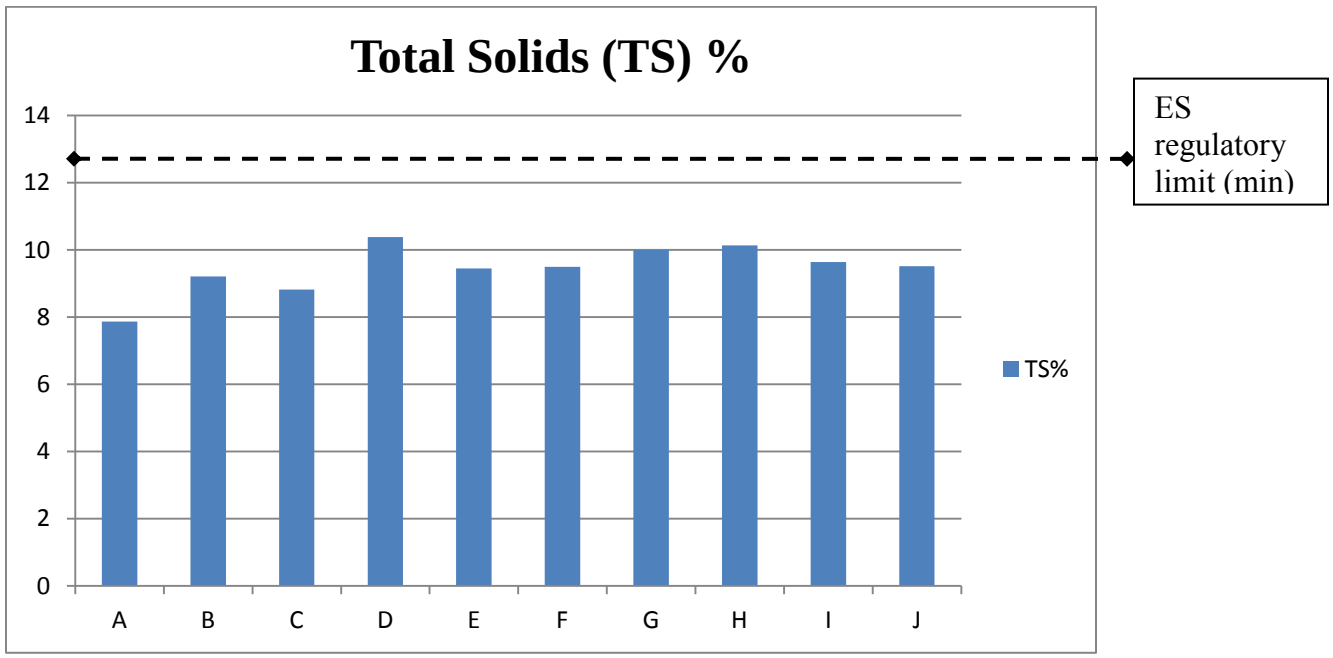
Table 10: Mean specific gravity of domestic pasteurized milk marketed in Addis Ababa

Pasteurized milk sample*	A	B	C	D	E	F	G	H	I	J	Overall Mean
Specific gravity	1.018	1.024	1.020	1.026	1.022	1.021	1.025	1.026	1.024	1.023	1.023

*Different letters represent different brands of pasteurized milk marketed in Addis Ababa

Addition of water dilutes milk reducing its total solids content. The mean specific gravity of the pasteurized milk samples observed in the present study was 1.023gm/ml (Table 10) which is lower than the recommended value range of 1.027 to 1.035gm/ml (Tamime, 2009). Therefore determination of total solids and solids not fat content is an important factor in judging milk quality in terms of added water.

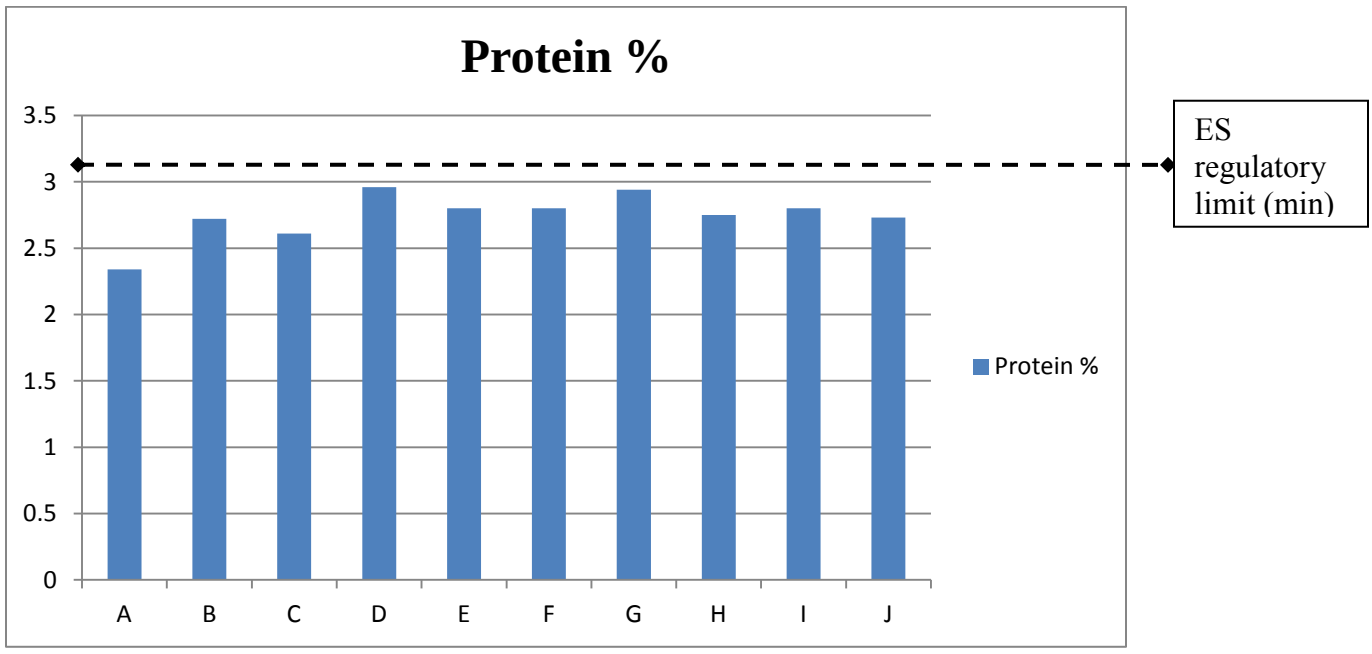
Figure 4: Comparison of total solids content of commercial pasteurized milk marketed in Addis Ababa against regulatory limit of Ethiopian Standard (ES)



*Different letters represent different brands of pasteurized milk marketed in Addis Ababa

The mean protein content of the ten pasteurized liquid milk samples was 2.75%, which is much lower than the minimum requirement set by the Ethiopian Standard of 3.20%. The highest protein content was found to be 2.96%, while the lowest value was 2.34%. The protein content of raw cow milk in Ethiopia has been reported to vary from 3.31 to 3.43% (Tola *et al*., 2007, Gemechu *et al*., 2015). This value is still lower than the claimed protein value of the pasteurized milk samples considered in the present study. While a protein value of 3.5% was written on the packaging material of those marketed products, none of the samples conform to even the minimum legal requirement of 3.2% let alone to satisfy their nutritional labeling that seems hardly reachable under the prevailing context. During pasteurization, the major nutrients are not altered and the process has little effect on the composition (Tetra Pak, 1995) and hence the lower value of the protein content of the pasteurized milk observed in the present study might be attributed to the use the raw material whole milk that had lower protein content or dilution of the milk with water.

Figure 5: Comparison of protein content of commercial pasteurized milk marketed in Addis Ababa against regulatory limit of Ethiopian Standard (ES)



*Different letters represent different brands of pasteurized milk marketed in Addis Ababa

The composition of milk varies considerably with the breed of cow, stage of lactation, feed, season of the year, and many other factors. However, some relationships between constituents are very stable and can be used to indicate whether any tampering with the milk composition has occurred (Ralph, 1998). Most of the major milk processors in and around Addis Ababa do not have their own dairy farms thus do not produce milk. They rather collect the raw milk from different small holder farmers and cooperatives located in the environs of the capital. Even those processors that have their own dairy herd depend on the surrounding smallholder producers for much of their daily milk intake. As reported by earlier studies, addition of water is commonly practiced in areas that are near to the capital city or have market access to the industries (Dehinenet *et al.,* 2013). This can be explained in the present study by lower than normal specific gravity of the pasteurized milk samples of 1.023 gm/ml. The specific gravity of normal milk ranges from 1.027 to 1.035gm/ml (Tamime, 2009). According to O'Connor (1993), the lower value of specific gravity of milk is indicative of addition of water. Therefore, the lower

value of the major nutritional constituents of pasteurized milk observed in the present study might be highly associated with the addition of water in the raw material milk.

In general, the contents of the major chemical compositions of the pasteurized milk samples considered in the present study do not meet the minimum standard set by the Ethiopian standard except for their fat contents.

7. Conclusion and recommendation

Based on the indicators considered in the present study, the quality of the pasteurized milk samples observed in the present study was of sub-standard. All pasteurized milk samples had total bacterial and coliform counts higher than the recommended standard and even most are positive for *E. coli*. The high bacterial count leads to early spoilage of the products before the expected shelf life, which makes it unfit for human consumption, and the presence of indicator organisms in high numbers can possibly lead to food borne illnesses. The coliform count is the index of sanitary quality of foods during handling and processing but all the pasteurized samples exceeded the maximum standard level of 10 cfu/ml. With regard to chemical composition the protein, total solids and solids not fat contents of all of the pasteurized milk samples were much below compared with the standard set and not in agreement with the nutritional label claimed in their package. The lower value of the major nutritional constituents of pasteurized milk observed in the present study might be highly associated with the addition of water in the raw material milk. Consumers have the right to get for what they pay for. From the above findings it may be concluded that none of the pasteurized milk samples conform to the standard with respect to chemical composition and bacteriological quality.

The presence of pathogenic organisms is harmful to human health. Therefore, frequent inspection of marketed pasteurized milk should be carried out to check whether they meet the minimum legal standards. The overall hygienic conditions surrounding the production and handling of milk should also be monitored. In this regard, the concerned governmental authority need to routinely conduct a hygiene audit and support the industries for corrective actions thereby ensuring the public health safety. Apart from improving their in-house hygienic standards, pasteurizing industries should also monitor the quality of incoming raw milk that has profound effect on the final product qualities. They should also strengthen their capacity of quality control to deliver safe and quality products to consumers. There is also lack of sufficient research with regard to pasteurized milk qualities that necessitates further detailed studies to address the problems and suggest solutions to the concerned stakeholders.

Appendix

MPN Table: For 3 tubes each at 0.1, 0.01, and 0.001 g inocula, the MPN per gram and 95percent confidence intervals

Pos. tubes			MPN/g	Conf. lim.		Pos. tubes			MPN/g	Conf. lim.	
0.10	0.01	0.001		Low	High	0.10	0.01	0.001		Low	High
0	0	0	<3.0	–	9.5	2	2	0	21	4.5	42
0	0	1	3.0	0.15	9.6	2	2	1	28	8.7	94
0	1	0	3.0	0.15	11	2	2	2	35	8.7	94
0	1	1	6.1	1.2	18	2	3	0	29	8.7	94
0	2	0	6.2	1.2	18	2	3	1	36	8.7	94
0	3	0	9.4	3.6	38	3	0	0	23	4.6	94
1	0	0	3.6	0.17	18	3	0	1	38	8.7	110
1	0	1	7.2	1.3	18	3	0	2	64	17	180
1	0	2	11	3.6	38	3	1	0	43	9	180
1	1	0	7.4	1.3	20	3	1	1	75	17	200
1	1	1	11	3.6	38	3	1	2	120	37	420
1	2	0	11	3.6	42	3	1	3	160	40	420
1	2	1	15	4.5	42	3	2	0	93	18	420
1	3	0	16	4.5	42	3	2	1	150	37	420
2	0	0	9.2	1.4	38	3	2	2	210	40	430
2	0	1	14	3.6	42	3	2	3	290	90	1,000
2	0	2	20	4.5	42	3	3	0	240	42	1,000
2	1	0	15	3.7	42	3	3	1	460	90	2,000
2	1	1	20	4.5	42	3	3	2	1100	180	4,100
2	1	2	27	8.7	94	3	3	3	>1100	420	–

Source: adapted from (the US Food and Drug Administration (2010) in Most Probable Number from Serial Dilutions

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