20

ASSESSMENT OF QUALITY AND CONSTRAINTS AFFECTING PRODUCTION TO CONSUMPTION OF MILK FROM PERI-ADDIS ABABA DISTRICTS OF OROMIA TO MILK RETAIL CENTERS IN ADDIS ABABA

BY

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A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES, COLLEGE OF NATURAL SCIENCESOF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF REQUIREMENT FOR MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

JUNE, 2014

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DECLARATION

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Lists of Abbreviations

ANOVA-Analysis of Variance

AOAC-American Official Association of Analytical Chemists

CC-Coli form Count

cfu- coli form forming units

CSA- Central Statistical Agency of Ethiopia

EHNRI- Ethiopian Health and Nutrition Research Institute

ENA- Ethiopian News Agency

ESAP- Ethiopian Society of Animal Production

EU- European Union

FAO- Food and Agricultural Organization of United Nations

ILRI- International Livestock Research Institute

LIA- Lysine Iron Agar

LSD- Least Significance Difference

masl- meters above sea level

ml- millimeter

TSA- modified Tryptone Soy Agar

TSI- Triple Sugar Iron Agar

MoA- Ministry of Agriculture

MSA-Manitol Salt Agar

^{o}c- Degree centigrade

^{O}F- Degree Fahrenheit

PDA-Potato Dextrose Agar

SFP-Staphylococcal Food Poisoning

SNV-Stichting Nederlandse Vrijwilligers(Netherlands Development Organization)

SPCA- Standard Plate Count Agar

SPSS- Statistical Package for Service Solution

TBC- Total Bacterial Count

UDSS- Urban Dairy Sector Study

USAID-United States Agencies for International Development

VRBA- Violet Red Bile Agar

WARDO-Woreda Agriculture and Rural Development Office

Abstract

The study was conducted at peri-Addis Ababa districts of Oromia with the aim to assess quality of milk at each critical point, and to identify knowledge gap, constraints affecting production to consumption chain of milk supply. A total of 102 milk producing farmers at Holeta, Sebeta and Sululta districts were selected by using multi-stage purposive sampling method. A total of 60 raw milk samples were collected hygienically from each presumed critical points and examined for their microbial and gross nutrient composition analysis. The main proximate and mineral values evaluated were ash, protein, fat, total solids and solid not fat; and Ca, Fe, Zn and P, respectively. The main microorganisms assessed were aerobic mesophilic bacterial count, Coli form count, and fecal coli form count, E.coli count, Salmonella, Staphylococcus species and Yeast and Mold Counts. About 99% of participants in the areas market whole milk and 94% of the milk produced per households was sold. About 96.1 and 23% of the participants stated that milk production and marketing in areas maintain household food security and profitable farm activity respectively. The major challenges of milk production and marketing in the areas were; feed shortage, high feed cost, disease, shortage of land for grazing, and price fluctuation during fasting season, long term contract for milk marketing and milk quality, respectively. Besides, lack of training for producers, lack of awareness on standard milk and milk product production and marketing, lack of aseptic milk handling and use of traditional flavor plants on milk microbial load were major knowledge gap in the areas. The mean ash content for Sebeta, Holeta and Sululta samples collected from farmers were 0.60±0.032, 0.77±0.025 and 0.50±0.007 mg/100gm, respectively. There was significant difference ($p<0.05$) in ash content among critical points and between districts. Besides, protein and fat have showed a significant difference among critical points($p<0.05$). The mean total bacterial count were: 6.48±1.065, 7.2±1.152, 7.02±0.169 and 6.7±0.694, 7.88±0.416, 7.20±0.056 log cfu/ml at farmer and retail shop of Sebeta, Holeta and Sululta, respectively. The overall mean coli form counts ranged from 5.42±1.735 to5.78±0.985; 5.53±1.034 to 5.63±0.625 and 4.18±1.228 to 6.35±0.435 log cfu/ml from farmer and retail shops of Sebeta, Holeta and Sululta respectively. E.coli was detected 26 (43.33%) of the samples at different critical points. Staphylococcus species was isolated from 17(28.33%) of samples collected from different critical points in the study sites. However, no Salmonella was found in all the samples. Mean value of yeast and mold counts were varied from 3.77±0.475 2.46±1.155, 2.16±1.259 and 3.45±0.261, and2.30±0.193, 2.99±0.824 log cfu/ml at farmer level of Sebeta, Holeta and Sululta respectively. Generally, the present was revealed that the gross nutrient composition of milk was in range of acceptable limit. However, it was contained higher microbial load than different standards and considered as substandard. Therefore, intensive study on microbial status of milk in the study sites should be conducted to assure safety and quality.

Key Words: *food safety and quality, food security, value chain, critical points, microbial load, knowledge gap, FS-AAS*

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1. Introduction

Ethiopia is believed to have the largest livestock population in Africa. Despite its huge population, the livestock subsector in the country is less productive in general, and compared to its potential, the direct contribution to the national economy is limited (Kedija *et al.,* 2008; Sintayehu *et al.,* 2008). Consequently, the national milk production and overall milk consumption in Ethiopia are very low, even compared with other African countries of lowest livestock population (Zegeye, 2003; Melese and Beyene, 2009).

For smallholder farmers, dairying provides the opportunity to the efficient land use, labor and feed resources and generates regular income (Yitaye *et al.,* 2009). In Ethiopia, one of the developing countries, urban and peri-urban dairying constitutes an important sector of the agricultural production system (Yitaye *et al.,* 2009).

Livestock represents major national resources and form an integral part of agricultural production system (Gebrewold *et al.*, 2000). Cows contribute to about 95% of the total annual milk produced at national level, while small ruminants and camels contribute 12.5% and 6.3%, respectively (Kedija *et al.*, 2008 and CSA, 2010). More than 75% of the product is absorbed locally for consumption (Getachew and Gashaw, 2001).

Dairy production, among the sector of livestock production systems, is a critical issue in Ethiopia where livestock and its products are important source of food and income, and dairying have not been fully exploited and promoted in the country (Sintayehu *et al.,* 2008). To be effective, the efforts to improve the productivity of smallholder dairy production and improve its market orientation needs to be supported and informed by detailed understanding of the current and dynamic condition of production, marketing, processing and consumption of milk and dairy products (Asfaw, 2009).

In the context of developing countries, the potential advantages of market-oriented smallholder dairying is improving the welfare of farm households and its multiplier effects on other sectors of the economy. Milk and milk products generates income for the farm households on regular basis, milk provides a highly nutritious food for people of all age groups and particularly for infants and lactating mothers thus reducing the problem of malnutrition among rural households and the value adding activities such as the

processing, marketing and distribution of milk and milk products also create employment opportunities in the rural and urban sectors (Bennet *et al.*,2006 and Asfaw, 2009).

Nutritionally, milk has been defined as the most nearly ''perfect food". It is a compensatory part of daily diet especially for the mothers with child as well as growing children (Javaid *et al.*, 2009; Olatunji *et al.*, 2012). It is daily produced, sold for cash or readily processed. It is a cash crop in the milk-shed areas that enables families to buy other foodstuffs, contributing significantly to the household food security. It also constitutes a significant proportion of the value of all livestock food products in Ethiopia (about 56%), while livestock food products constitute an important proportion of the value of total food products in the country (Belete *et al.,* 2010).

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the its physico-chemical properties, it needs strict hygienic condition to avoid contamination of milk with microorganisms. Therefore, the microbial content of milk is a major feature in determining its quality (Rogelj,2003). Food quality and safety standards in Ethiopia are one of the most concern areas because producers need to minimize loss while the general public would like to have a fair idea of what standard of food to buy for consumption. Also the safety of the food supplied for consumption especially for foods like milk is of paramount concern.

Microbial load is a major factor in determining milk quality. It indicates the hygienic level exercised during milking, cleanliness of the milk utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animals (Ahmed, 2009; Fatine *et al.*, 2012).

The initial microbiological quality of milk can vary substantially based on factors such as the health of the animal, the sanitary condition of the milking environment and milker (Biruk *et al.*, 2009). Microbial contamination of milk can therefore originate from within the udder; the exterior of the teats and udder; and from the milk handling and storage equipment (Biruk *et al.*, 2009; Negash *et al.*, 2012).

Unsafe milk not only impairs with public health but also its perishable nature makes it most susceptible to spoilage organisms that could result in quantitative loss of the milk. Hence, the quantitative loss of meager resource milk, due to spoilage could affect not only the small holder milk producer but also the consumption by urban dwellers and the entire nation. A range of factors can lead to food being unsafe, such as poor handling and storage conditions, naturally occurring toxins in food itself, contaminated water, pesticides and drug residues, and lack of adequate temperature control. Such safety problems, in extreme cases, can have negative impact on the food security status of a country (FAO, 2011).

FAO (2011) defines food loss as the decrease in edible food mass throughout the supply chain which could have a significant impact on the livelihoods of many smallholders given that most of them live on the margins of food insecurity. These losses can occur at production, postharvest and processing stages in the chain (Parfitt *et al.*, 2010). For milk, losses at agricultural production level refer to decreased milk production due to unhealthy dairy cow and its environment. At postharvest handling and storage, milk loss is caused by mishandling and degradation during transportation between farm and distribution. The quality of milk may be lowered by numerous factors such as adulteration, contamination during and after milking and the presence of udder infections (Esron *et al.,* 2005).

Seventy percent of total milk sold in Addis Ababa informally comes from smallholder dairy production system located around Addis Ababa. The raw milk is thus marketed directly or through middlemen without any form of pasteurization or quality control measures (Ashenafi, 2002; Zelalem and Faye, 2006). Hygienic production and safe handling of milk from the production to consumption chain has always been a matter of consumer complaint on the ground that the milk is presumed sub standard. This could partly be attributed to non-existence of dairy facilities at small holders' production system. Usually milk is collected in a milk collection container, before loading to centers of processing or milk retail shops.

Awareness and knowledge of available standards for dairy products, processing, handling and marketing is not well ahead. One can presume that milk at the spot of immediate production may neither be sub-standard nor adulterated. Most of the concern of quality and safety are raised as milk starts along the supply chain.

Hence, care to be taken in favor of quality and safety as of individual milk coming to the collection center. Therefore, it raises the concern that the consumers are having and thus calls for thorough scientific investigation. Possible questions can be raised at this junction. Is the milk produced around-Addis Ababa really sub standard? If then, what factors negatively affected the standard which could have been met by the milk? Can these factors conned to alleviate the problem so that the milk will meet the standard? Obviously, the research questions to be answered will enable producers to add value, consumers will get confidence of not to be impaired with their health.

1.2 Statement of the problem

Due to the highly perishable nature of milk coupled with mishandling practices from production up to the consumption stage, the amount produced is subject to high post harvest losses. Estimated post harvest losses of up to 40 % of milk and its derivatives have been reported from milking to consumption (Felleke, 2003).

Post harvest losses and quality deterioration are mainly attributed to mishandling in the dairy chain from farm to fork. According to FAO (ENA, 2004), the value of annual milk and dairy product losses due mainly to mishandling across five African and the Middle East countries (Kenya, Tanzania, Uganda, Ethiopia and Syria) was over US $90 million.

Numerous epidemiological reports have implicated that non-heat treated milk and raw-milk products as the major factors responsible for illnesses caused by food-borne pathogens. Cross-contamination with pathogenic microorganisms can gain access to milk either by faecal contamination or by direct excretion from the udder into milk (El-Ziney and Al-Turki, 2007). Consequently, not only the immediate disposal of milk and the medical costs that contribute to food insecurity but also that the productive labor gets unable to produce could negatively affect the potential for food security.

Safe consumption of dairy products play headstone role in the human nutrition both interms of satisfying the nutrient requirement and ensuring individual household food security by providing daily food crop purchasing power for the individual and generating income for individual household respectively.

However, due to its high pershiability and conducive media for growth of microorganisms, most of dairy products are exposed to microbial contamination during transportation and storage which results in loss of products, economic loss to the producer and food-borne illness. If there are no systematic study will not be possible and no control measures to manage potential microbiological hazards, raw milk and products made from raw milk can present a high level of risk to public health and safety.

Before pasteurization was introduced, dairy products including drinking raw milk were frequently implicated in many different food-borne illness outbreaks. In addition to pasteurization, milk production, transport and dairy processing businesses are required to control potential food safety hazards associated with their business by implementing documented food safety programs.

Meager report related to milk production and consumption in Ethiopia categorically had been related to the stage of development of the country. For instance, Yilma (2006) reported that hygienic practices during production, processing and handling of milk and milk products in the central Ethiopia are substandard. However, there is scanty information on the microbial properties and composition of raw milk in Ethiopia (Eyasu and Fekedu, 2000; Zelalem and Faye, 2006). Such reports coupled with notion of problems related to milk supply chain and detection of milk microbial in dairy products after transportation and storage in the of Peri-Addis Ababa to milk retail in the city calls for systematic study and research project of remedy for the malady.

1.3. Objective of Study

1.3.1. General objective

The general objective of study was to evaluate the quality of milk and associated constraints, and more over to examine possible knowledge gap of standards critical points of milk supply chain from Peri-Addis Ababa districts of Oromia to the market of Addis Ababa.

1.3.2. Specific objectives

1. To evaluate safety and microbial load from production to retail distribution of milk in the study areas
2. To assess knowledge gap of standards and supply of milk in the study area
3. To identify constraints affecting consumption of standardized milk products in study area
4. To assess gross nutrient composition of milk at each critical point

1.4. Significance the study

Since there was meager study on the safety/quality and microbial load after transportation of dairy products from peri-Addis Ababa to shopping centers and processing, value chain of products from production to consumption after transportation specially in the study area till now, it will be a baseline for researchers and consumers and selling centers of the products significantly. This study will provide significant information on transportation effect, level of microbial load and post harvest handling of milk and its products in the study area.

It will contribute for helpful to identify the gap and undertake further study on safe dairy product transportation, handling especially at retail level in the area. Moreover, this study will help nutritionists, food scientists and Publish the information to the end users as stakeholders of supply chain from Peri-Addis Ababa districts of Oromia to Addis Ababa. Possibility of milk spoilage will be minimized which consequently reduce quantitative loss and better way of milk distribution for consumption will be enhanced.

2. Literature Review

2.1 Overview of milk production in Ethiopia

Ethiopia holds the largest livestock population in Africa estimated to be about 52.13 million cattle, 24.2 million sheep and 22.6 million goats. However, total national milk production remains among the lowest in the world, even by the African standard (Zelalem 2006, Elias *et al.*, 2008 and CSA, 2012). The total annual national milk production in Ethiopia comes from about 10 million milking cows and is estimated by 3.2 billion liters that is, 1.54 L/cow on average (Kedija *et al.*, 2008 and CSA, 2012). The bulk of this milk production (81.2%) comes from cows, while small ruminants and camels contribute 12.5% and 6.3%, respectively (Kedija *et al.*, 2008).

The total annual milk production in Ethiopia increased at a rate of 1.2% for indigenous stock and 3.5% for improved stock(Redda, 2003). The per capita consumption of milk in the country is 16kg/year(Saxena *et al.,* 1997). Hence, about 6 million tones of milk are required per annum to feed the population as per the standard (Saxena *et al.,* 1997). In order to meet the growing demand in Ethiopia, milk production has to grow at least at a rate of 4% per annum(Azage,2003).

In Ethiopia, around 97-98% of the annual milk production is accounted by the traditional milk production system. Generally, the milk production system in East African countries in general and Ethiopia in particular is dominated by smallholder dairy production system(Zelalem,2006).

Milk production is a critical issue in Ethiopia; a livestock-based society-where livestock and its products are important sources of food and income, and dairying has not been fully exploited and promoted. The annual milk production is estimated about 3.8 billion liters from cattle and 165 million liters from camel (CSA, 2013).

2.2 Consumption Pattern of Cow Milk in Ethiopia

Milk Consumption in Ethiopia shows that most consumers prefer purchasing of raw milk because of its natural flavor (high fat content), availability and lower price. Specific upper income market segments prefer and can afford packaged processed milk.

Packaging costs alone may add up to 25% of the cost of processed milk depending on the type of packaging used. Polythene sachets of processed milk are cheaper alternatives (SNV,2008).

An increasing number of people are consuming raw unpasteurized milk (Oliver *et al.*, 2009). The consumption of raw milk and its products is common in Ethiopia (Zelalem, 2003), which is not safe from consumer health point of view as it may lead to the transmission of various diseases. Raw or processed milk is a well know food medium that supports the growth of several microbes with resultant spoilage of the product or infections (intoxications) in consumers (Oliver *et al.*, 2005).

Ethiopia has a low level of milk consumption compared to other countries in the region (Kenya = 90 lt/cap; Uganda = 50 lt/cap). Even though Ethiopia has the largest inventory of milk producing animals,(cattle, sheep, goats and camels), per capita consumption of milk is low compared to Kenya and Sudan those having fewer livestock. The national per capita consumption of milk and milk products is estimated at 17 kg (Ahmed,2009). Per capita income levels in Ethiopia place it in the range with Tanzania and Rwanda with annual per capita consumption of milk at less than 20 kg.

2.3 Challenges of milk production

The milk production is constrained by many factors affecting the milk subsector in pastoralists were low milk productivity, low quality milk, poor organization of development actors in the sub sector and in the chain, lack of business orientation among the pastoralists, lack of market oriented producer organization and lack of poor market infrastructure. The causes of these factors could be categorized into natural, institutional and social. The effect of all these factors on the milk subsector resulted not only decreasing the milk production from time to time but forced the pastoralists to operate the milk and milk products business at loss which consequently trapped them to live in a vicious circle of poverty (Worku *et al.*, 2012).

Ulfina *et al.*,(2013) reported marketing constraints include fluctuation in demand and supply of dairy products (as a result of feed shortage and different socio cultural reasons), poor infrastructure (lack of cooling facilities, simple processing equipments and quality

testing skills and equipments) and the long time fasting of the members of the Ethiopian Orthodox church which coupled with absence and or very limited milk processing facilities in the area.

2.4 Milk Marketing Chain

Market oriented dairy farming is regularly subjected to various constraints ranging from poor animal health, inadequate farm management to deficient marketing of milk and milk products (Tebug *et al.,* 2012). Facilitating market participation of households as well as developing chain competitiveness and efficiency are valuable preconditions to improve livelihoods (Lundy *et al.,* 2004; Padulosi *et al.*, 2004).

Dairy production, among the sector of livestock production systems, is a critical issue in Ethiopia where livestock and its products are important source of food and income, and dairying have not been fully exploited and promoted in the country (Sintayehu *et al.* 2008).

Value chain approach starts from an understanding of the consumer demand and works its way back through distribution channels to the different stages of production, processing and marketing (GTZ, 2006). Production is the basic segment for any value chain analysis and it is the pivotal point that makes the value chain to develop and attain competitiveness. The improvement made in this level of the chain could have a significant implication in enhancing competitiveness in all other levels of the chain. It holds true particularly for agricultural value chains in general and milk and milk products in particular.

Unless farm households adjust to rapidly changing markets which are characterized by quality and food safety, vertical integration standards and product traceability, reliability of supply, there will be a risk of competitiveness and inefficiency for the entire value chain (Vermeulen *et al.,* 2008).

The dairy value chain comprise about 500,000 smallholder rural farmers who produce about 1,130 million liters of milk of which 370 million liters of raw milk, 280 million liters of butter and cheese and 165 million liters is consumed by the calves. The remaining 315 million liters was marketed through both informal and formal retailers through farmers' organizations (Mohammed, 2009).

Informal milk marketing accounts for over 70% of total milk sales in Addis Ababa and accounts for the majority of urban dairy farm milk production. Raw milk is marketed locally by smallholder urban producers directly or through middlemen. Raw milk is generally produced in very unhygienic conditions, it is after adulterated, and can transmit zoonotic diseases which presents a public health risk. Formal milk marketing of 17 pasteurized milk and milk products accounts for under 30% of total milk sales in Addis Ababa even though these products are hygienically prepared and considered safe for human consumption (UDSS, 2006).

A study showed that in the Addis Ababa milk shade there are about 66,766 cattle and 31,062 (46.5%) are estimated to be crossbred dairy animals. The main milk suppliers to the city are urban dairy farmers in Addis Ababa and peri-urban dairy producers located around the city in Oromia and Amhara Regions. The estimated annual milk production from these two sources is 49,505 tons and 5,005 tons, respectively, totaling 54,510 tons (Gete *et al.*, 2007).

2.5 Composition of Cow Milk

Chemically, milk is a complex mixture of fat, protein, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water, make it a complete diet (Haug *et al.,* 2007). In addition to the positive impact that dairy and livestock can have on household income, assets, and food security, the nutritional significance of dairy products has also been well documented.

Milk is also highly energy dense, which is important for young children or chronically ill with lack of appetite. Importantly, milk comprises all eight essential amino acids, thus constituting high quality protein. Research has demonstrated the positive nutritional impacts of dairy, including an association between increased consumption of milk and improved child growth (Zhu *et al*, 2004; Hoppe *et al*,2004).

Milk contains protein and minerals essential to promoting the growth and maintenance of human life during the three periods of life: child hood; providing protein, minerals and fat to support the body's development, during the adolescence; offering conditions for a rapid

growth building consistent muscles, bones and endocrine and for elderly people it represents a source of calcium essentially to maintain the integrity of bones (IEA, 2007).

Milk and milk products are main constituents of the daily diet, especially for vulnerable groups such as infants, school age children and old age (Enb *et al.*, 2009; Li-Qiang *et al.*, 2009). It is an ideal source of macronutrients such as calcium (Ca), Potassium (K), and phosphorous (P) and micronutrients such as copper (Cu), iron (Fe), Selenium (Se), and Zinc (Zn). These are known to be essential for normal growth and are co-factors in many enzymes and play an important role in many physiological functions of man and animals (Li-Qiang *et al.*, 2009).

Milk is considered as a nearly complete food since it is a good source for protein, fat and major minerals (Enb et al., 2009). Milk is an important source of all basic nutrients required for mammals including human beings. Milk from various mammals such as cow, buffalo, goat, sheep is used for different nutritional purposes, e.g. feeding to young ones and preparation of some nutritional products such as milk cream, butter, yogurt, sour milk, etc. (Hassan, 2005).

Negash *et al.* (2012) from East Shoa and West Arsi Zones of Oromia reported 5.5, 9.1and 3.5, for fat, Solid-Not-Fat and protein respectively from raw cow milk collected from individual households. Similarly, Rehrahie and Yohannes (2000) obtained 5.9% fat and 9.3% SNF, but a little higher than 2.7% protein. The other study by Alganesh *et al.* (2007) indicates 6.1% fat, 3.3% protein and 8.2% solid-not-fat.

On top of this, Zelalem *et al.* (2004) reported solid-not-fat, fat and protein percents, indicated 8.4, 5.4 and 3.2%, respectively from Ethiopian Boran cows. The acceptable range of fat and protein from cow milk reported by O'Connor was between 2.5 to 6.0% and between 2.9 to 5.0% for fat and protein respectively (O'Connor,1994).

According to Ramesh (2006), the major components of milk are water (87.4%), milk solids (12.6%), solids-not-fat (9.0%), fat (3.6%), protein (3.4%), milk sugar or lactose (4.9%) and ash or minerals (0.7%). The constituents may vary with genetic (breed and individual cow and variability among cows within breed),feed type and environment (interval between milking, stage of lactation, age, feeding regime, disease and completeness of milking).

2.6 Mineral Elements in raw cow milk

Calcium is responsible for many regulatory functions, such as normal cardiac rhythm maintenance, blood clotting, hormone secretion, muscle contraction and enzyme activation (Cashman, 2002). Milk and dairy products (cheese and yogurt) are very rich sources of calcium. The majority of dietary Ca (70 %) comes from dairy products because in milk, casein micelles constitute the natural vector of Ca (Canabady-Rochellea and Mellemab, 2010).

Iron occurs in two forms such as heme and as non-heme iron. Heme iron present as hemoglobin and myoglobin is absorbed directly as intact iron porphyrin complex. Heme iron is well absorbed (15-35%) and little influenced by physiological or dietary factors (Monsen *et al.,* 1978)

The intake of non-heme iron varies widely and, is affected by dietary components and iron status of an individual. Iron deficiency in complementary food is one of the most common global nutrition disorders both in all developed and developing countries. Approximately 50% of children and women of reproductive age and about 25 to 30% of men are iron deficient in developing countries. Iron deficiency even in the absence of anemia can have adverse functional consequences particularly for cognitive development and behavior in children(Yip, 1994).

According to the report of USDA (2008), the mineral elements specially calcium, phosphorus and iron in raw cow milk in mg/100gm were 113, 91 and 0.03 respectively. Dawd *et al.* (2012) reported average concentrations of the mineral element Zn and Fe were (4.92±0.28mg/kg),(1.21±0.077mg/kg) respectively for raw cow milk samples collected from selected sub-cities in Addis Ababa. Ghada (2005) reported the main mineral elements from raw cow milk in Egypt; Ca, P, Zn and Fe 119±0.690, 95.03±0.72, 0.38±0.00 and 0.070±0.02mg/100g, respectively.

Table1:Concentration of major mineral elements in mg/100gm in goat, sheep and cow milk compared to human

	Milk			
Mineral element	Goat	Sheep	Cow	Human
Calcium (mg/100 g)	106-192	136-200	107-133	22-41
Phosphorus (mg/100 g)	92-148	80-145	63-102	12-17
Magnesium (mg/100 g)	10-21	8-19	9-16	3.0-3.4
Potassium (mg/100 g)	135-235	174-190	144-178	46-55
Sodium (mg/100 g)	34-50	29-31	40-58	12-15
Chloride (mg/100 g)	100-198	71-92	90-106	32-49

Source: Coni A etal,1999; park,2006; Deutchen Forschungsanstalt fur Lebensmittelchemie,2012

2.7 Microbial Load of Cow Milk

The rapid growth of microorganisms, particularly at high ambient temperatures can cause marked deterioration and spoiling the milk for liquid consumption or process into milk products. This can be avoided by adopting the simple, basic rules of clean milk production (Lore *et al.* 2006).

Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, feaces and grass. The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health (Coorevits *et al.* 2008).

The initial microbiological quality of milk can vary substantially based on factors such as the health of the animal, the sanitary condition of the milking environment and the milker (Biruk *et al.*, 2009) Microbial contamination of milk can therefore originate from within the udder; the exterior of the teats and udder; and from the milk handling and storage equipment (Biruk *et al.*, 2009).

Milk adulteration, poor hygiene, malpractices, lack of preservation technology, cooling facilities and sanitation conditions are the main causes of loses in quantity and quality of milk (Haasnoot *et al.*, 2004).

Smoking of milk equipments was found to lower microbial load (Almaz, 2001). Unhygienic production of milk and milk products and improper storage, cause the early spoilage with microorganisms (Nanu *et al.*, 2007).

Dairy factories usually receive the milk directly from the producer or from mobile and fixed milk collection centers. This process may contribute to increase in the microbial load (Imami Meybodi *et al.*, 2010). In order to provide safe and healthy milk products, the Hazard Analysis and Critical Control Points (HACCP) system should be implemented starting from milk collection, through processing and storage (Cannas and Noordhuizen, 2008).

Leitner *et al.* (2008) established that refrigerated storage of good-quality milk from a single cow resulted in moderate deterioration of its quality, low level of bacterial growth (standard plate and psychrotophs counts), and low small losses of curd yield. When milk was collected from farm bulk milk tanks and from dairy silos, its quality deteriorated faster than that of single-cow milk resulting in high bacteria counts.

The presence of bacteria in milk can cause some reduction in the raw milk quality and certain bacteria contaminants with their associated enzymes and toxins may even survive pasteurization and create health hazards (Oliver *et al.,* 2005).

Contamination of milk not only reduces the nutritional quality but also consumption of such milk threatens health of the society (Karmen and Slavica, 2008). High microbial counts in raw milk are responsible for quality defects in pasteurized milk, UHT processed milk, dried skim milk, butter, and cheese (Barbano *et al.,* 2006).

Additionally, selecting raw milk of high quality has been associated with a decrease in consumer complaints caused by fluid milk quality (Keefe and Elmoslemany, 2007). Earlier research conducted in Ethiopia revealed that the microbial counts of milk and milk products produced and marketed in the country are generally much higher than the acceptable limits.

Zelalem (2010), in his study on the microbial properties of marketed milk and milk products sampled from 10 dairy potential areas in the country reported a similar

observation and mentioned that microbial counts in samples of whole milk, *Ergo* and skimmed milk were particularly higher. Raw or processed milk is a well-known good medium that supports the growth of several microbes with resultant spoilage of the product or infections/ intoxications in consumers.

Milk from a healthy udder contains few bacteria but it picks up many bacteria from the time it leaves the teat of the cow until it is used for further processing. These microorganisms are indicators of both the manner of handling milk from milking till consumption and the quality of the milk. Milk produced under hygienic conditions from healthy animals should not contain more than 5×10^5 bacteria count/ml (O'Connor, 1994). The lower counts of bacteria may be due to good cleaning system and good handling from farms to the plant (Chye *et al.*, 2004).

Quality milk means, the milk which is free from pathogenic bacteria and harmful toxic substances, free from sediment and extraneous substances, of good flavor, with normal composition, adequate in keeping quality and low in bacterial counts (Khan *et al.*, 2008).

The bacterial contamination of milk not only reduces the nutritional quality but also consumption of such milk threatens health of the society (Karmen and Slavica, 2008). Total number of organisms in milk as disease causative agent in relation to its proper evaluation for consumption is important. The notable disease causing bacteria in milk are *Salmonella*, *Brucella*, *Staphylococcus*, *Listeria*, *E. coli* and *coli forms*. *Coli forms* and *E. coli* are normal inhabitants of the large intestine and their presence in milk could indicate fecal contamination (Olfa *et al.*, 2013).

Common bacterial contaminants of milk (milk borne pathogens) are *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella spp.*, *Klebsiella pneumoniae, Proteus spp, Campylobacter jejuni,* and *Bacillus cereus* (Isabel, 2009 and Shekhar, 2010).

2.7.1 Total Bacterial Count

Estimation of bacterial content is a commonly used procedure to measure the quality of milk. The Standard plate count (SPC) method is a worldwide standard method to estimate the total bacterial numbers present in raw milk (Smiddy *et al.*, 2007; Ahmed, 2009; Fatine *et al.*, 2012).

It is indicated that total bacterial count is a good indicator for monitoring the sanitary conditions practiced during production, collection, and handling of raw milk (Chambers, 2002). The acceptable limit for total bacterial count in raw cow milk according to American and European community member states is between 2×10^5 and $4x10^5$ *cfu/ml* (APHA, 1995).

The total bacterial count is an indicator of the general hygienic condition during milk production, transportation and storage. In general, lack of knowledge about clean milk production and use of unclean milking and handling equipment might be some of the factors which contributed to the poor hygienic quality of milk, diseased udder and unfavorable storage temperature (Worku *et al.*,2012, Biruk *et al.*, 2009 and Tola, 2002).

Unclean hands of workers, poor quality of milk, unhygienic conditions of the manufacturing unit and water supplied for washing the utensils could be the source for accelerating the bacterial contamination of milk products beside the post manufacturing contamination (Elmahmood *et al.*, 2007). Poor milk handling practices during milking, poor animal health services, and use of poor potable water which were linked to markedly high total bacterial count (Nandy *et al.*, 2007).

The SPC is an estimate of the total number of viable aerobic bacteria present in raw milk. This test can also be used to monitor herd health and production sanitation since consistent application of proper milking practices, udder hygiene and good mastitis prevention and control practices should allow dairy producers to routinely produce milk with a low SPC (<5000*cfu/ml*). Most farms can produce milk with counts of <10,000 *cfu/ml*. High bacteria counts (>10,000 *cfu/ml*) suggest that bacteria are entering milk from a variety of possible sources. The most frequent cause of high SPC is poor cleaning of milking systems (Jayarao *et al.*, 2004).

According to an earlier report, milk samples collected from smallholder producers in East Wollega (Southern Ethiopia) had average total microbial count of 7.60 log *cfu/ml* (Tola, 2002). The total aerobic bacterial count (TABC) of milk obtained from dairy farms ranged from 1.88×10^4 to 3.15×10^6 *cfu/ml* with an average value of 5.84 *cfu/ml* and the total aerobic bacterial count of milk samples obtained from milk vendors ranged from 3.5×10^7

to 2.05×10^{10} *cfu/ml* with an average value of 9.14 ± 0.9 *cfu/ml* (Tesfay *et al.*, 2013) and the overall mean total bacterial count of cows' milk at farm level was 7.58 log *cfu/ml* at Bahir Dar Zuria District (Tassew and Seifu, 2011).

A total bacterial count ranging from 6.0 to 8.8 log *cfu*/ml in which 15% of the samples had a count equal or above 7.7 log *cfu/ml* in raw milk samples in Southern Ethiopia was also reported (Beyene, 1994). In another report, milk sampled from most of the dairy cooperatives operating in the country had total bacterial count of 108 *cfu*/ml (Francesconi, 2006). These values are much higher than the acceptable limits in different countries (104 to 105 *cfu*/ml). This implies that the sanitary conditions in which milk has been produced and handled are substandard subjecting the product to microbial contamination and multiplication (IFCN, 2006).

The total bacterial count of cows' milk produced in Bila Sayo and Guto Wayu districts of eastern Wollega to be 7.4 x10 and 2.0 x 10 *cfu/ml*, respectively (Tola,2002). The mean total aerobic bacterial count and coli form count of raw milk were 7.07 log10*cfu/ml* and 1.82 log 10*cfu/ml*, respectively and the total bacterial load obtained from raw milk sample was 1.82 log10*cfu/ml* (Mosu *et al.*, 2013).

2.7.2 Coliform Bacteria

Coliform counts can indicate fecal contamination or contamination from equipment that has not been properly cleaned and sanitized (Schmidt, 2008; Bintsis *et al.*,2008; Biruk *et al.*, 2009). *Coli form* count above 500 cell/ml indicates poor hygiene either during equipment cleaning or between milking with common contaminants such as bedding, manure, soil or water (Murphy and Boor, 2003). The acceptable limits of *Coliform* counts in milk should be less than 100 cell/ml (Douglas, 2003; Shojaei and Yadollahi, 2008).

Milking udder with sub-clinical mastitis and wet environment lead to contamination of bulk tank milk and hence raw milk reaches the consumers with elevated Coli form count (FAO, 2008; Zadoks *et al.*, 2007). Therefore, the higher coli form count observed in general, could be due to the initial contamination of the raw milk samples either from the cows, the milkers, milk containers and the milking environment.

The higher count in milk could be attributed to the substandard hygienic conditions practiced during production and subsequent handling Counts of *Enterobacteriaceae* and coliform were higher than acceptable limits: *Enterobacteriaceae* <1 and coliform <10 *cfu/ml* for pasteurized milk and coliform <100 *cfu/ml* for raw milk intended for direct consumption (Council Directives 92/46/EEC, 1992).

Zelalem and Bernard in Ethiopia found higher *Coli form* count in raw milk samples under study which could be due to the initial contamination of the milk samples from the cow's milk, the milkers, milk containers and the milking environment (Zelalem and Bernard, 2006). Kagki *et al.* (2006) showed that in addition to faecal contamination, other factors such as milking wet udders, inadequate cooling of milk and udder infection are the main sources of *Coli form* in bulk milk.

Tassew and Seifu (2011) reported mean coli form count of 4.49 *cfu/ml* in milk sampled from around Bahir Dar and Mecha districts. Another study showed higher coli form count by Zelalem and Faye (2006) with counts of 6.57*cfu/ml* for cows' milk collected from different producers in the central highland of Ethiopia.

According to a report from Eastern Wollega, Ethiopia, raw cow's milk sampled from smallholder producers contained coliform counts of about 4.46 log *cfu*/ml (Tola, 2002). Similar counts were also observed in raw milk sampled from smallholder producers in the central highlands of Ethiopia (Zelalem and Faye, 2006; Zelalem *et al.*, 2007a). Higher counts of different species of *Enterobacteriaceae* were reported with *Escherichia coli* being the most abundantly isolated species (Zelalem *et al.*, 2007), which is a good indicator of recent fecal contamination (Bintsis *et al.*, 2008).

Coli form counts of more than 100 cell/ml suggests poor hygienic practices (Jayarao and Wolfgang, 2003). Khan *et al.* (2008) reported a count between 300-400 cell/ml and counts of more than 600 cell/ml reported in the summer market milk. Vendor milk is more contaminated with coliform bacteria compared to milk from the shops (Adil and Iman, 2011).

Tesfay *et al.* (2013), reported the mean value coliform count of raw milk samples collected from dairy farms in Dire Dawa, Ethiopia was 4.13±0.757 log10 *cfu/ml*; Ombui *et al.* (1994) reported that about 50% of the samples collected from milk collection centers in Kenya were contaminated with about 2.7*cfu/ml.*

2.7.3 E. Coli

Raw milk exposed to untreated and contaminated water, cattle or human faeces can easily be contaminated with *E. coli*. Unpasteurized milk and dairy products made from raw milk (such as soft cheese) act as vehicles for transition of *E. coli* to human (Dweik *et al.*, 2012).

Consumption of raw milk is a high risk behavior leading to morbidity and mortality (Keene, 1999). Generally, raw milk consumption is a traditional practice among farm families (Jayarao *et al.*, 2006). The presence of *E. coli* organisms in milk and milk products is an indication of unsanitary production and\or improper handling of either milk or milk utensils (El-zubeir and Ahmed, 2007 and Olfa *et al.,* 2013).

Recovery and counting of *E. coli* is used as reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard. *E. coli* is onc of the main inhabitants of the intestinal tract of most mammalian species, including humans. Most *E. coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in human (Kaper *et al.,*2004).

Contamination of milk and milk products with pathogenic bacteria is largely due to processing, handling, and unhygienic conditions. *E. coli* is one of the bacteria that exist in the normal micro flora of the intestinal tract of humans and warm blooded animals. *E. coli* is, furthermore, a known causative agent of diarrhea and other food-borne related illnesses through the ingestion of contaminated foodstuffs (Olfa *et al.,*2013).

Presence of *E.coli* in milk products indicates the presence of enteropathogenic microorganisms, which constitute a public health hazard. The incidence of the species of *E. coli* itself in milk and milk products, as a possible cause of food-borne illness, is not significant if *E. coli* is normally a ubiquitous organism, yet the pathogenic strains if present could be harmful to consumers (Hahn,1996).

Presence of organisms in the pasteurized milk is indicative of unhygienic consumption. It has been shown that contamination of milk to *E. coli* in the milk distributing centers is increasing, which is indicative of the unhygienic conditions in preparing, distribution and transportation (Olfa *et al.,* 2013). *E. coli* frequently contaminates food and it is a good indicator of fecal pollution. Enteropathogenic *E. coli* can cause severe diarrhea and vomiting in infants, and young children (Benkerroum *et al.,* 2004).

Out of 60 isolates, 21(12.6%) of isolates were confirmed as *E. coli* (six from curd and 15 from cottage cheese); out of 63 isolates, 12 isolates were confirmed as *S. aureus* (from cottage cheese), on the basis of morphological and biochemical characterization. According to these results a higher contamination with *E. coli* and *S. aureus* was found in cottage cheese as compared to curd (Singh and Prakash, 2008).

In other study Endale *et al.* (2013), reported 44.4% of *E. coli* positive samples from different critical points of milk production at Mekelle and (Olfa *et al., 2013*) found that 13 out of 50 milk samples (32.5%) were contaminated with *E. coli* at Sfax, Tunisia from unpasteurized raw milk samples collected from different localities. The majority of the Coliform isolates from the raw milk consumed in Khartoum state were *Escherichia coli (*32%), *Enterobacter* species (29.2%)(Adil and Iman, 2011); Vahedi *et al.* (2013) in 42 (42%) from raw cow milk. Tesfay *et al.* (2013), reported average mean value of *Escherichia coli* count of raw milk samples collected from dairy farms were 3.64±0.776 *cfu/ml.*

2.7.4 Staphylococcus species

Staphylococcus aureus is a Gram-positive, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus. The *Staphylococcus* genus is subdivided into 32 species and subspecies (Montville and Matthews, 2008).

Staphylococcus aureus produces staphylococcal enterotoxin (SE) and is responsible for almost all staphylococcal food poisoning (Montville and Matthews 2008; FDA, 2012). *S. aureus* is a ubiquitous organism frequently isolated from raw milk manually draw from individual animals, bulk raw milk and naturally, from milk of dairy cattle suffering from mastitis.

Staphylococcus intermedius, is commonly associated with dogs and other animals, can also produce SE and has been rarely associated with staphylococcal food poisoning (Loir *et al.*, 2003).

Staphylococcal food poisoning (SFP) is one of the most common food borne illness worldwide with high occurrence second to salmonellosis (Aycicek *et al.*, 2005). It is often associated with the ingestion of manually handled foods that contain one or more highly heat stable staphylococcal enterotoxins(SEs). Many foods will support growth of staphylococci and toxin production. Milk, dairy products and meats, especially handled foods, are common vehicles that are frequently implicated in Staphylococcal food poisoning (Smith, 2007).

Food handlers carrying *enterotoxin*-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination via direct contact or through respiratory secretions (Argudin *et al.,* 2010). In proper drawn milk, the typical counts of *S. aureus* are 100-200 *cfu*/ml. In the case of a contaminated udder, the counts may increase up to 10^4 *cfu*/ml (Asperger and Zangerl, 2003).

Milk and dairy products are frequently contaminated with enterotoxigenic *Staphylococcus* species, which are often involved in SFP, especially in areas characterized by a high level of consumption of these products, since staphylococci are often involved in cases of subclinical mastitis of ruminants resulting in contamination of milk (Salandra *et al.*, 2008). Hence, raw milk is a potential source of staphylococci in milk and milk products, especially in the case of mastitic milk and defective pasteurization (Kaloreu *et al.*, 2007).

Illness through *S. aureus* range from minor skin infection such as pimples, boils, cellulites, toxic shock syndrome, impetigo, and abscesses to life threatening disease such as pneumonia, meningitis, endocarditis, and septicemia (Soomro *et al.,* 2003).

Aydin *et al.* (2011), reported the occurrence of Enterotoxigenic *S. aureus* in food samples collected from retail markets and dairy farms in Turkey and was found in 11.3% of meat, 10.2% of unpasteurized milk, 8.0% of dairy products, 3.5% of bakery products and 2.3% or ready-to-eat products; and according to Vahedi *et al.* (2013) *Staphylococcus aureus* was observed in (22%) of milk samples collected at the dairy farms and super markets in Sari city;

Mekuria *et al.* (2013) reported prevalence of (15.5%) *S. aureus* out of the total samples examined from selected dairy farms around Addis Ababa; Mekonnen (2009) identified prevalence of *Staphylococcus* as 33% and 46% from buckets milk and tanks milk, respectively, from Debre Zeit.

Hempen *et al.* (2004) from Gambia, reported 33.3% of the raw milk samples showed counts of coagulase-positive *Staphylococci spp.* above $2x10^3$*cfu*/ml, this milk would not have been accepted by European standards for the production of milk products.

2.7.5. Salmonella

Salmonella is an enteric bacteria and is the most common food-borne pathogen (Weigel *et al.*, 2004; Mizumoto *et al.*,2005). *Salmonella* are mostly facultative anaerobes, oxidase-negative, catalase-positive and gram negative rods. Most strains of Salmonella are motile and ferment glucose with production of both acid and gas. The disease called Salmonellosis is caused by member of *Salmonella. Salmonella* have several species. *Salmonella enterica* is the most responsible for 99.9% infection in humans and most of the infection are zoonotic in origin (Yan *et al.*,2003).

Salmonella is a leading cause of food borne illness (White *et al.*, 2001). Globally, more than 93 million cases of gastroenteritis are caused by non typhoidal *Salmonella* with 155,000 deaths each year. Of these cases, 80.3 million cases were estimated to be food borne. Salmonellosis is a common intestinal illness caused by numerous *Salmonella serovars* with clinical manifestations that vary from severe enteric fever to mild food poisoning (Jones *et al.*, 2004) and intestinal illness in animals (Radostits *et al.*, 2007).

According to Jayaroo and Henning (2001), *Salmonella* was isolated from 6.1% of bulk tank milk sample from dairy herds in South Eastern Dakata and Western Minnesota.
In other study conducted in Addis Ababa salmonella was isolated from 2.1% of milk samples collected from different supper market in Addis Ababa (Tesfaw *et al.*, 2013). Tesfay *et al.(2013),* reported *Salmonella* spp. with a percentage of 18.8% and 41.7% for milk samples obtained from dairy farms and vendors, respectively from Dire Dawa. Van Kassel *et al.* (2004) reported a 2.6% occurrence of *salmonella* spp. in raw milk samples collected from US dairies.

2.7.6 Yeast and Mold Counts

Moulds are important in milk, which is used for the manufacture of cheese and other dairy products. The presence of wild types of moulds is undesirable as they may influence the organoleptic characteristics of the cheeses, they can produce mycotoxins and represent a potential health risk (Wouters, *et al.,* 2002).

Karmen and Slavica (2008) in Slovania reported the mean yeast and mold counts of 1.9 log10 and 2.3 log10 *cfu/ml*, respectively in raw cow milk collected from transportation bulk of at the entrance of dairy farm; Fadda *et al.* (2004) reported the mean yeast count 2.64 log10 *cfu/ml* in raw milk from farms located in different areas.

Ahmed (2011) reported, yeast and molds were detected in 28 samples with the mean and maximum values of 6.1 and 7.4 log *cfu/ml*, respectively, from camel milk collected from Meiso districts of Oromia in different stage of lactation.

3. Materials and methods

3.1. Description Study Area

The study was conducted in three peri-Addis Ababa districts (*Sululta, Holeta and Sebeta*) of Oromia Regional States of Ethiopia. The study sites were selected based on their milk production potential as well as their lion's share to milk retail market at Addis Ababa. Sululta is located between 9°4'30"N to 9°30'59"N and 38°31'26"E to 38°58'49"E. Although it is geographically located in North Shewa zone of the Oromia Regional State, Sululta district has been administratively placed under the Oromia Special Zone surrounding Finfine. Animal production system is mainly mixed crop-livestock type of farming system (CSA, 2004).

Holeta is situated at a distance of 31 km West of Addis Ababa and located at 9°02' N latitude and 38°29' E longitude in Oromia National Regional State (ONRS) of Ethiopia. It is found at an average altitude of 2449 m a.s.l. The area is one of the major dairy potential sites in Oromia Regional State Sebeta is located 24km from South west of Addis Ababa at a latitude and longitude of 8°55'N38°37'E and an elevation of 2356 masl. These areas take the lion's share in terms of their milk production potential and contribution to Addis Ababa milk market. The supply chain followed in this study, therefore, is from production sites in these districts to Addis Ababa milk retail market place.

The main agricultural practices of the study areas are mixed, crop-livestock production system, in which *Teff*, wheat, lentil and chickpea are widely grown. Agriculture is strictly rain fed. The areas' rainfall and temperature ranges between 800-1500mm $year^{-1}$ and 10-25°c, respectively. Animal products, especially dairy products, play a headstone role in household food security both by direct consumption and purchasing of other food items in the area (WARDO, 2012).

3.2. Sampling procedure

In the first step, diagnostic survey was made and discussions were held with agricultural extension officers and available dairy cooperatives/unions in the three districts. First hand information was gathered regarding the overall frame of dairy production, milk handling mechanisms, pattern of consumption or milk transportation, to market places in Addis

Ababa. Also the hygiene condition and safety management of milk supply chain was observed.

The information obtained from the respective district offices of agriculture was used to select focal villages (Kebele administrations) and individual farmers following multi-stage purposive sampling technique. Two villages were selected purposively from each district on the basis of dairy production potential, linkage to milk market, access to supply milk collection center, presence of dairy cooperative unions and accessibility. Subsequently, a total of 102 dairy farmers (40 from Holeta, 30 from Sululta and 32 from Sebeta) were selected with the help of Development Agents and used as study participants. In addition to milk producing households, collection centers, informal merchants and dairy cooperative union at each districts were interviewed referring to milk marketing outlets, handling patterns and transportation of products to further processing and final consumers.

3.3. Survey study

Following the routes, milk retailers' in Addis Ababa were also interviewed on milk handling, transportation, cooling system and if they met long-term consumer's milk demand and preference. Both qualitative and quantitative data were collected using multiple subject formal survey by pre-tested, semi-structured questionnaire. Key informant interviews of zonal and districts' agricultural offices, dairy cooperative chairmen of respective districts and secondary information were also used to assess consumers' habit of dairy product consumption, their knowledge, preference and associated constraints regarding production, purchasing and consumption of the milk from Agricultural Offices, dairy cooperative union and retail shop.

A semi structured questionnaire was prepared in English and translated into Afaan Oromo for easy administration and back to English to check consistency. Then pre-tested semi-structured questionnaire was used to generate data. A total of 102 dairy stakeholders were selected as study participants on the questionnaire and for the interview. Respondent interviewee included household milk farmers, milk collection center, dairy union, informal merchants and retail shops from production to final retail distribution.

Survey parameters were collected on household characteristics, herd structure, major challenges of milk production in the area, utilization pattern, milk collection and transportation, milk handling and marketing along the value chain.

3.4 Sample Collection and Preparation

Two-hundred fifty ml of raw milk samples were collected from different milk value chain following Hazard Analysis and Critical Control Points. Samples were collected aseptically in sterile sample bottles in a cold ice box with ice bag at temperature <6°c. A total of 60 duplicate raw cow milk samples were collected from different milk marketing channels i.e. farmers, milk collection centers (MCC), informal merchants (IM), Milk Cooperative Unions (MCU) and milk vendor/retail shops (MVS) in the study area.

The samples were taken to the laboratories of Ethiopian Public Health Institute (EPHI) and Center of Food Science and Nutrition of AAU for chemical & microbiological analyses. Microbial analyses were done within 6 hrs of collection. For chemical analyses, milk samples were put in refrigerator for overnight, transferred to a deep freeze at -40^{0}c for 24 hrs and then freeze dried. Dried samples were coded & transferred to brown bottle and put in desiccators having silica gel as desiccant & put at moisture free place until proximate and mineral analyses.

3.5. Chemical Analysis

3.5.1. Determination of Fat

Ether extract as an estimate of crude lipid was determined using Soxhlet extraction apparatus by exhaustively extracting a known weight of sample by diethyl ether. Two gram of dried sample was weighed in duplicate run and covered with fat free cotton at the bottom and top was weighed in extractor thimble (W1). A clean, dried round bottom extraction flask containing a few granules of boiling chips were weighed (W2). The extraction thimble and flask was fitted on the extractor unit and 60 ml of diethyl ether was dispensed automatically into the flask using a tube connected on the top of the extraction unit. Condenser was connected to the Soxhlet extractor and cold water circulation was put on.

The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 4 hrs. The solvent was recovered and the oil dried in an oven set at 70 °C for 1 hr. The round bottom flask and oil was cooled in desiccators and then Weighed (W3) (AOAC, 2005) official method 989.05.The ether extract was calculated as:

Weight of fat (Wf) = Wa-Wb

Where; *Wa* = Weight of extraction flask after extraction

Wb = Weight of extraction flask before extraction

Crude fat content (g/100) = (Wf [100 - moisture, %]/Wd)

Wd = Dried sample obtained after determination of moisture

3.5.2. Determination of crude protein

The protein content of the samples was determined on the basis of total nitrogen content by micro Kjeldahl method of crude nitrogen determination(AOAC, 2005) using the official method 991.20. A digestion flask containing about 0.5 g of dried sample, was digested in a 100ml Kjeldahl digestion flask by boiling with 6mL of concentrated H_2SO_4 and 3grams of Kjeldahl digestion tablet (selenium: potassium sulphate mixture) as a catalyst and boiling point raising agent. 3.5ml of 30% hydrogen peroxide was added to the digestion mixture after which the teccator tube containing the mixture was set with teccator digestor. The digestion continued for 4 hours at 370 °C until the mixture became a clear solution.

The digested sample solution was made up to 50 ml with distilled water and 30 ml of 40% sodium hydroxide. The solution was slowly and automatically added to the mixture by Kjeldhal titration apparatus. The ammonia liberated was collected in 30 ml of 1% boric acid solution containing a mixed indicator. Steam was applied to the solution to distill out ammonia evolved with the distillate collected into the boric acid solution. Ammonia was estimated by titrating with standard 0.1N HCl solution. Blank nitrogen determination was carried out in a similar manner and subtracted from the sample nitrogen. Crude protein was determined by multiplying the value obtained for percentage nitrogen content by a factor of 6.38.

$$\%N_2 = \frac{14 \times M \times V_t \times V_{100}}{\text{Weight of sample (mg)} \times V_a}$$

$$\%\text{Crude Protein} = \%\ N_2\ (\text{Nitrogen}) \times 6.38$$

Where, M = Actual molarity of Acid

V = Titer value (Volume) of HCl used

V_t= Total volume of diluted dig

V_a = Aliquot volume distilled

3.5.3. *Determination of Total Solid*

To determine the total solids, five grams of milk sample was placed in a pre-weighed and dried duplicate of crucibles. The samples were kept at 102^0C in a hot air oven overnight. Then, the dried samples were taken out of the oven and placed in desiccators. Then the dry sample was weighed (O'Connor, 1994).

$$\textit{Total solid} = \left[\frac{\textit{crucible Wt + oven dried sample wt-Crucible Wt}}{\textit{Sample Wt}}\right] x\ 100$$

3.5.4. *Determination of Solids- not -fat*

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

SNF= (TS-fat) x100

3.5.5. *Determination of Ash*

The freeze dried milk samples were analyzed for ash and mineral contents. Crucibles were washed with distilled, deionized water and boiling with 6N HCL on hot plate and rewashed with distilled water, then dried at 100°c for 1hr then put into desiccators for 30 minutes for cooling then weighed(W1). Freeze dried 1gm duplicate milk sample was weighed &transferred to a pre-weighed crucible (W3). Charred on the hot plate until organic matter was fully removed under hood, then burn-off at Muffle Furnace at 550°C for 1hr then taken out and rinse with a drop of distilled water by using pasteur pipette Again put on hot plate till dry off and again to Muffle Furnace at the same temperature for 30 minutes.

Then taken out, rinse with deionized water and 5 drops of concentrated HNO_3 and dried on hot plate at low temperature and then put again into Muffle Furnace at the same temperature for 30 minutes. Finally taken out and put into desiccators for 30 minutes for cooling and weighed (W2) (AOAC, 2005).

Calculation; % Ash = (W2-W1)*100/W3

Where: W3-weight of fresh sample
W2- weight of crucible and dried sample
W1- weight of empty dried crucible

3.6. *Determination of Mineral Element*

AOAC (2005) was used to estimate minerals. Accordingly, all the crucibles required for minerals analyses were washed with 6N HCl and glass wares with 10% nitric acid. The required number of crucibles was placed in an oven for 30 minutes at 100°C, cooled in desiccators for 30 minutes and weighed (W1). One gram of samples were accurately weighed and subjected to chare at hot plate starting from low temperature under a hood.

The samples were ashed in a muffle furnace at 550°C for 1 hour and the crucibles were taken out from the furnace, cooled, and moistened with a few drops of deionized water. The water was evaporated on a hot plate. The samples were ashed once more for 30 minute at 550°C and cooled in the crucible; some drops of deionized water and 5 drops of concentrated HNO_3 were added and evaporated on hot plate as described above. Finally, the samples were ashed as above for 30 minutes at the same temperature as previously described. The crucibles were cooled in desiccators for 45 minutes and then weighed (W2). Six ml of 6N HCl was added to the ashed sample to wet it completely and carefully taken to dryness on a low temperature hot plate. Seven ml of 3N HCl was added and the crucible heated on the hot plate until the solution just boils.

Then the solution was cooled and filtered through a Whatman No 1 filter paper into a 50 ml graduated flask. Five ml of 3N HCl was added to the crucibles and the solution was heated until it starts boiling, cooled and filtered into the graduated flask. The crucibles were washed at least three times with deionized water and the washings were filtered into the flask. 2.5ml of lanthanum chloride solution per 50 ml of solution was added to the extract to free bounded calcium. The content of the flask was cooled and diluted to the volume of the flask with deionized water.

The sample extract solution was transferred to polyethylene bottle and stored until used for determinations of minerals. Blank was prepared without sample by taking the same amount of reagents under the same condition.

The minerals, *viz.* calcium, iron, and zinc were analyzed using Shimadzu atomic absorption spectrophotometer (AA-6800/ "AA Wizard" software). Phosphorous was determined using UV-visible spectrophotometer (CECIL Instruments, Cambridge England, deuterium F 500mA, power T3. 15A) based on AOAC (2005) method 970.39. Briefly, 1ml of the digested solution for mineral analysis was taken into 100ml volumetric flask and the volume was filled to the mark with deionzed water. From the aliquots, 5 ml of the solution was taken into test tubes in a duplicate to which 0.5 ml molybdate was added and homogenized. Aminonaphtholesulphonic acid (0.2ml) was added to the mixture and mixed. Series of standards were prepared in a similar manner and the mixture was left to stand for ten minutes. Absorbance of standard, blank and samples were read at 660 nm using UV Visible Spectrophotometer. Absorbance versus concentration calibration curve was constructed and the equation obtained was used to calculate the unknown phosphorus concentration in the samples.

$$\text{Phosphorus in mg/100gm} = \frac{(A_S - A_B) * \text{dilution factor} * \text{extracted volume} * 100}{\text{Slope} * \text{weight of sample} * 1000}$$

Where, A_S= absorbance of sample

A_B= absorbance of blank

Slope= from the calibration curve

3.7. Microbial Analysis

3.7.1 Aerobic plate Count

Serial dilutions of sample up to 10^7 on peptone water was done by 250ml flask containing 25ml milk sample and 225ml peptone water biological and shake using digital shaker to have uniform distribution of microorganisms for 2-5 minutes. Sterile Petri-dish was prepared and 1ml of diluted sample was poured into Petri-dish and molten PCA(Plate Count Agar) was poured into Petri-dish and allowed to stand till it solidify and incubated at

30°c for 72hrs in inverted direction and result count by using colony counter (Richardson, 1985).

3.7.2. Total Coli form counts

Twenty-five ml of milk sample was diluted by 225ml of peptone water in 250ml volumetric flask and 1ml of diluted sample was poured to a Petri-dish and molten TSA was poured to encourage the growth of stressed microorganisms during transportation and allowed to solidify for 10 minutes, then molten VRBA(Violet Red Bile Agar) was poured to Petri-dish and allowed until it solidify and incubated at 37°c for 24 hrs in inverted and count by using colony counter. Then confirmed by transferring suspected colonies to Brilliant Green Bile broth with inverted drum's tube by taking 5 colonies from each petri-dish by checking gas production after 48hrs (Richardson, 1985).

3.7.3. Fecal Coli form count and E.Coli

Twenty-five ml of milk sample was diluted by 225ml of peptone water in 250ml volumetric flask and 1ml of diluted sample was taken and poured to sterile Petri-dish and molten TSA was poured to encourage stressed microorganisms during transportation and allowed for 30 minutes. Then molten Violet Red Bile Agar was poured to the petri-dish and allowed 10 minutes for solidifying and incubated at 44.5°c for 48hrs in inverted and colony count by using colony counter. Then confirmed by transferring on to E.C broth with inverted drum's tube by taking 5 colonies from each petri-dish and incubated 24hrs at 44.5°c for gas production due to fermentation of lactose. Then those having gas production on E.C broth were transferred to nutrient broth by taking loopful from E.C broth, incubated at 44.5°c for 24hrs in order count E. coli and taken out and wait for 5 minutes at room temperature then 0.5ml of Kovac's reagent was added and checked for dark red color at top of test tube.

3.7.4. Isolation and characterization of *Staphylococcus* species

Twenty-five ml of milk sample was diluted by 225ml of peptone water in 250ml volumetric flask and 1ml of diluted sample was taken and pour on sterile Petri-dish and molten MSA was pour on the petri-dish and allow to solidify for 10 minutes and incubated at 37°c for 24hrs in inverted and checked for fermentation, sub cultured until pure colonies

were observed and transferred to TSA for 24hrs at 37°c. Then sub cultured until pure colony observed and biochemical tests was undertaken for confirmation were Gram's staining, catalase test using hydrogen peroxide, Coagulase test by rabbit plasma, blood Agar by using 7% sheep blood in blood base agar for checking hemolytic, finally DNase test was conducted(ISO, 1999 and Quinn *et al.*, 1999).

3.7.5. Isolation and characterization of *Salmonella* species

Pre-enrichment in non-selective media (25ml milk and 225ml Peptone saline) solution in 250ml flask was incubated at 37°c for 24 hrs for primary enrichment. After pre-enrichment in Bacteriological Peptone Water, 1 ml of culture was transformed into a tube containing 10 ml of Selenite Cysteine broth for secondary enrichment. Selenite broth was incubated at 37°C for 24hrs. Salmonella-Shigella (SS) Agar and Xylose Lysine Deoxycholate (XLD) medium (Oxoid) were used for plating purposes. A loopful of culture from selective enrichment broth was streaked separately on to each of the solid media and incubated at 37°C for 24 hrs. Then it was checked for formation of dark red colonies after 24hrs of incubation. Those plate having dark red colonies were sub-cultured and incubated again until having pure colony. Then it was transferred to further biochemical tests i.e. a single colony was taken and streaked into urea slant and incubated at 37°c for 24 hrs.

Then those samples urease negative after 24hrs incubation were suspected for Salmonella positive and then the samples were transferred into nutrient broth and checked for turbidity after incubating at 37°c for 6hrs. Then transferred into Simon's citrate, Lysine Iron Agar(LIA), TSI(Triple Sugar Iron)agar, Indole test and motility tests were undertaken to confirm pure isolate of Salmonella species by taking a sterile glass pasteur pipette and streaked at the slant then incubated at 37°c for 24hrs except for motility media which was first streaked at the center of media with glass pipette without touching the base of test tube and then streak at the slant and incubated at 37°c for 24hrs(ES ISO, 6785:2001).

3.7.6. Yeast and Molds counts

One ml of dilution was pour on Petri-dish and molten PDA(Potato Dextrose Agar) was pour on Petri-dish, allowed it solidify for 30 minutes and then incubated at 25°c for 7 days and count by using colony counter.

Then confirmed by streaking a loopful colony on slide smearing carefully by saline solution under 40x magnification lens microscope from each petri-dish(Richardson, 1985).

3.8 . Statistical analysis

Data were analyzed using SPSS software (ver.16, 2007) package. Descriptive statistics such as mean, frequency distribution and percentage was used to report data from survey study. The microbiological analyses data were transformed to logarithmic scales (log_{10}cfu/ml) and both gross nutrient composition and microbiological data analyzed as per one way ANOVA model. Significant log mean differences were separated based on Least Significant Difference (LSD) test mean separation technique. Means were declared significant at ($p<0.05$).

4. Result and Discussion

4.1. Household characteristics

Majority of the study participants were male (68.6%) and the remaining (31.4%) were females. The result was different from reports from other rural areas. This may be due to male hired laborers in milk producing households for performing different activities regarding to milk production. The result was different from Teshager *et al.* (2013) reports from Aba Illu Bora Zones of Oromia. Majority, 56.7 % (n=58) of study participants were in the age range 25-45 years, 42.2% (n=43) were in the age range of 46-70 years and 0.7% (n=1) were in the range of 71-95 years.

4.1.1. Herd structure

About 19.6%(n=20) of the participants own local dairy cows. However, majority of the participants own cross breed dairy cows. The mean number of cross breed and local cows were 2.54±0.169 and 2.50±0.174, respectively, per households on the study sites. About 43.2% of the participants have more than three milking cows, 18.3% own three milking cows, 17.9% own two dairy cows and 20.6% own only one dairy cow per households. This implies that milk production is one of important income generating activity in the areas and contributes greatly to household food security and economy.

4.1.2. Hygienic practices

Hygienic practices are major pathways to produce safe and quality products for the consumers there by reduces microbial contamination and loss of product. Table 2 indicates major hygienic practices followed by milk producers in the study sites. Source and type of water used for washing hand and utensil have profound effect on microbial contamination of the milk. About 26.5, 6.9, 46.1, 2.9 and 17.6% of the participants only used cold pipe water, warm river water, warm pipe, cold river water and cold well water, respectively for washing udder and teat before milking in the whole study site (Table 2).

Additionally, through hand washing (especially in the developing countries) in between milking, during pre-milking and post-milking stages by using safe disinfectants can enhance the safety of fresh milk (Oliver, 2005).

However, none of them wash hands before and after milking. On top of this, only 77.2% of the study participants wash their hands before milking in all the study sites. The proportion was higher at Sebeta then Holeta 77.2 and 76.5%, respectively. This might be due to lack of training for producers and other milk handlers on the washing of their hands and milk utensils that mitigate the growth of microorganisms and maintaining the safety of products thereby enhancing the safe product available for consumers and reduce the loss of product that have profound effect on food security.

Table 2: Percentage hygienic practices of dairy farmers followed during milking at different study sites

	Districts		
Hygienic practices	Sebeta n=32	Sululta n=30	Holeta n=40
Practicing barn cleaning daily	94.4	95.7	98.6
Using bedding materials for milking cows	26.6	63.4	78.6
Producers followed during milking			
Washing udder before and after milking	--	--	--
Washing udder before milking only	82.5	86.3	93.3
Not common practice	3.7	3.2	--
Some times	13.2	10.5	6.7
Washing hands before milking	77.2	70.9	76. 5
Type of water used for udder washing			
Cold	28.1	20.0	37.5
Warm	59.7	70.0	50
Both alternatively	9.7	-	8.3
***Sources of water for farm* activities**			
Warm tap/Pipe water	76.7	73.6	79.0
Well water	4.6	1.2	2.0
River water	18.7	25.1	19.0

Majority of participants did not use bedding materials for milking cows in the whole study areas. But the proportion was very low for Sebeta which was related to high price of material and unavailability.

Only 26.6, 63.4 and 78.6% of the respondents at Sebeta, Sululta and Holeta, respectively, use bedding materials. Use of bedding materials and frequent cleaning of barn have profound effect on reducing microbial contamination of teat and udder(Sintayehu *et al.*, 2008).

According to study participants, about 40% uses traditional flavoring agents and anti-microbial effect for cleaning milk transporting equipments. Among them about 22.5% and 20.6% used 'woira' and 'Kosorot' respectively and the remaining used 'Ajekis' and 'Largo' for washing equipments.

Almost all of the participants in the study area use plastic materials for milking, storage and transportation of milk and only insignificant number of participants;1.2% and 1.3% used metal can and stainless steel respectively and 1.1 % used clay pot for storage before transportation.

Table 3: Percentage milking procedure and frequency of dairy farmers followed during milking at different study site

	Districts		
	Sebeta	Sululta	Holeta
Pre-milking procedure	n=32	n=30	n=40
Use of towel for drying udder			
Common towel for cleaning and drying udder and teat	48.1	51.3	72.2
Individual towel for each	3.4	4.5	3.8
Massage with bare hand	64.4	59.1	50.3
No washing and drying	3.5	10.0	12.4
Milking procedure			
Hand milking	100	100	100
Machine milking	--	--	--
Milking Frequency			
Once daily	2.1	2.6	2.0
Twice a daily	96.3	96.8	97.3

Almost all participants households in the study sites follows milking their cows per day, (91.2%)morning and afternoon, (6.9%)morning only and (1%) milk cows either mid day, evening or morning. The result of present study was similar to that of Sintayehu *et al.* (2008) who stated majority of the participants (96.3%) milk their cows twice daily in Shashsmane-Dilla area, Southern Ethiopia.

4.1.3. Milk production per households and milking practice

Milk production and marketing have a significant effect on the household food security as well as contributing to the national GDP. Table 4 indicates milk production per household.

Table 4: Mean number of milking cows per/household and milk produced per study sites

Variables	Districts		
	Sebeta n=32	Holeta N=40	Sululta N=30
No. of cows currently milked			
One	8(27.5)	10(24.5)	8(24.1)
Two	6(22.5)	7(16.7)	6(17.2)
Three	5(18.6)	7(17.2)	11(29.6)
More than three	9(31.4)	12 (29.8)	10(28.8)
Amount of milk produced/day			
1-5 liters	1(2.9)	1(3.4)	1(3.1)
6-10 liters	11(38.6)	15(37.3)	13(36.9)
>10 liters	14(52.0)	20(49.1)	18(53.4)
>15 liters	2(6.5)	4(10.2)	2(6.6)
Use of cooling system			
Refrigerator	1(3.3)	1(1.6)	1(2.6)
Traditional system	11(40.0)	20(49.1)	17(49.6)
At room temperature	16 (56.7)	19(49.3)	16(47.2)

The mean number of cow from which milk is pooled daily was 2.59±0.114 per household in the whole study areas (Table 4). Majority of participants in the study areas pool milk from more than three cows (31.4%), from two cows(22.5%), from three cows(14.1%) and the remaining were from only one cows(20.7%).

About 52% of the participants in study sites produce on average more than 10 liters of milk daily and 45.1% and 2.9% of participants respectively produces 6-10 and 1-5 liters of milk per day/cow.

This implies that majority of study participants produce and market high amount of milk that helps to sustain their household food security. Consumption of milk at household level was very low and majority of milk was sold per households that helps to generate income. On the contrary to the present finding, another study Teshager *et al.* (2013) found higher mean (96%) of milk consumption per household.

About 96.1% of the participants intended to expand milk production for the future while the remaining were not interested to do so. About (96.1%) and (2.3%) of the participants, respectively responded that milk production maintains household food production and generates income/ profitable.

4.1.4 Milk handling practices

Major factors that affect quality of dairy products are related with type and hygienic status of milking utensils used as well as method and frequency of cleaning udder, storage of milk and transportation utensils. About 98, 97.1 and 94.15% of the participants in the study sites used plastic utensils for milking, storing before transportation and transporting milk.

The result of present study was higher than that reported by Sintayehu *et al.* (2008) in Southern Ethiopia. Besides, significant number of respondents use plastic jar having narrow neck which may not be suitable for cleaning and may cause for microbial growth. More than half of the study participants did not use aroma producing plants like woira (*Olea africana*) that have profound effect on reducing growth of microorganisms (Sintayehu *et al.*, 2008 and Asfaw, 2008). On the other hand, some participants use 'Ajekis' and Largo 'liquid soap' for washing utensils.

4.1.5 Milk production and household food security

Food security is alarming issue in worldwide currently. In its broad term food security describes safety, quality and enough food for all members of household to maintain productive and healthy life. Majority of participants in the area responded that milk production and marketing have a key role in maintaining household food security and nutritious diet to all household members.

About 52%, 45.1% and 2.9% of the participants produced more than 10 liters, 6-10 liters and 1-5 liters of milk per households per day on average, respectively. This indicates that the areas were potential for milk production and it contributes significantly to household food security.

About 67.3% of the whole participants in three districts participants that milk production and marketing plays invaluable role in household food security. From the total participants about 51.2%, 17.1 and 22% stated that milk production used as source of purchasing food crop, students school fee and saving bank, respectively.

At household level, females play great role in milking, milk handling and marketing of milk. About 47.5%, 12.9% and 39.6 female, male and both gender, respectively, of the participants declared that involvement in milk production in all districts.

4.1.6 Milk production and marketing in area

Marketing system of milk at study area is unorganized and is carried out through direct sellers (milk passes directly from the producer to the consumer) and indirect marketing channels where several agencies operate between producer and consumer. The channels in marketing of milk involved in this area include direct sellers, milk collection centers, informal merchants, milk cooperative unions, hotels, dairy product processing plants and retail shops. However, majority of the participants brought their milk to the collection center and private dairy processing plants.

Almost all of the participants were marketing milk travelling on foot by holding milk and small number of the participants were supplying milk by travelling by horse cart and others are by using bicycle. That was in line with Kedija *et al.* (2008), who reported majority of participants were market milk travelling on foot by holding milk in Meiso districts of Oromia.

Majority of milk was marketed to collection centers in the case of Holeta and Sululta and then to Addis Ababa where as in the case of Sebeta, majority of milk taken to private milk processing plant, collection center to Addis Ababa and informal merchants contribute to higher share of milk marketing outlets.

About 68.6 % and 31.4% of the participants bring milk twice daily to collection center and private processing plant, respectively. About 99% of the participants were marketing milk in the form of whole milk. Whereas Teshager *et al.* (2013) reported that traditionally selling of raw milk was considered as taboo and none of the respondents were involved in raw milk marketing in Algie, Oromia regional state, Ethiopia.

The result of current the study for milk marketing was higher than that reported by Teshager *et al.* (2013) in Ilu Aba Bora zone in that only 10.5% overall milk was marketed which indicates that milk production is the major income generating activity in the area that helps to maintain household food security. But the results of current study agreed with that of Agza *et al.* (2013) that showed about 94% of milk produced was sold while 6% was retained for home consumption, that shows the producers provide good service to the community in the area by serving as a good source of milk supply.

4.1.7. Major challenges of Milk production and marketing in the study areas

Milk production is one of crucial income generating activity that maintains household food security and national economy as whole. However, it is challenged by a number of factors that hinder level of production as well as safety issues of the product. As indicated in the figure1, the major challenges identified in the study sites include; feed shortage, high price of feed, disease, lack of capital, price fluctuation/market condition, and shortage of land for expansion.

Almost all of the participants were claiming feed shortage and high price of feed resource as the major challenge in the areas. Similarly, different research works Agza *et al.* (2013); Teshager *et al.* (2013); Kedija *et al.* (2008) in different parts of Oromia were implicated that milk production in Ethiopia is highly hindered by one or more of the above mentioned factors that affect productivity of milking cows as well as household income from them.

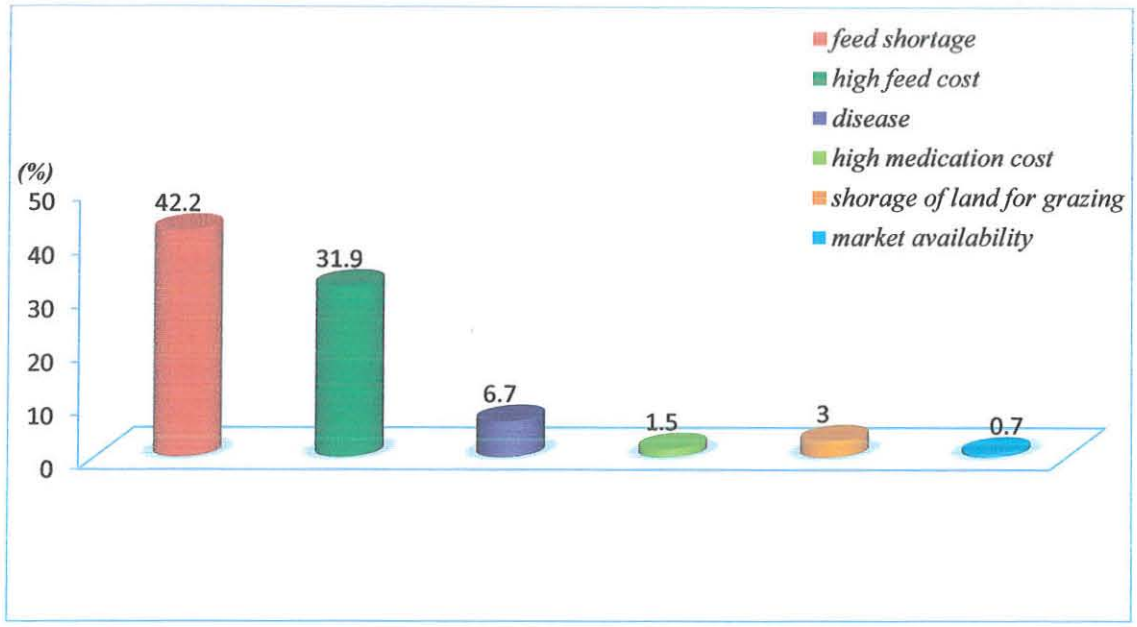


Figure1: The major challenges of milk production in the study site

On top of the above factors that challenge milk production in the areas, the milk produced also doesn't reach point of final consumption at required time and condition of product that creates conducive environment for growth of many microorganisms that spoil products and results in food safety hazard as well as loss of products. Major problems of milk marketing in the area identified were indicated in Figure 2 and include; price fluctuation during fasting months, distance to selling centers and/or market, long term contracts, milk quality, lack of quality based pricing system.

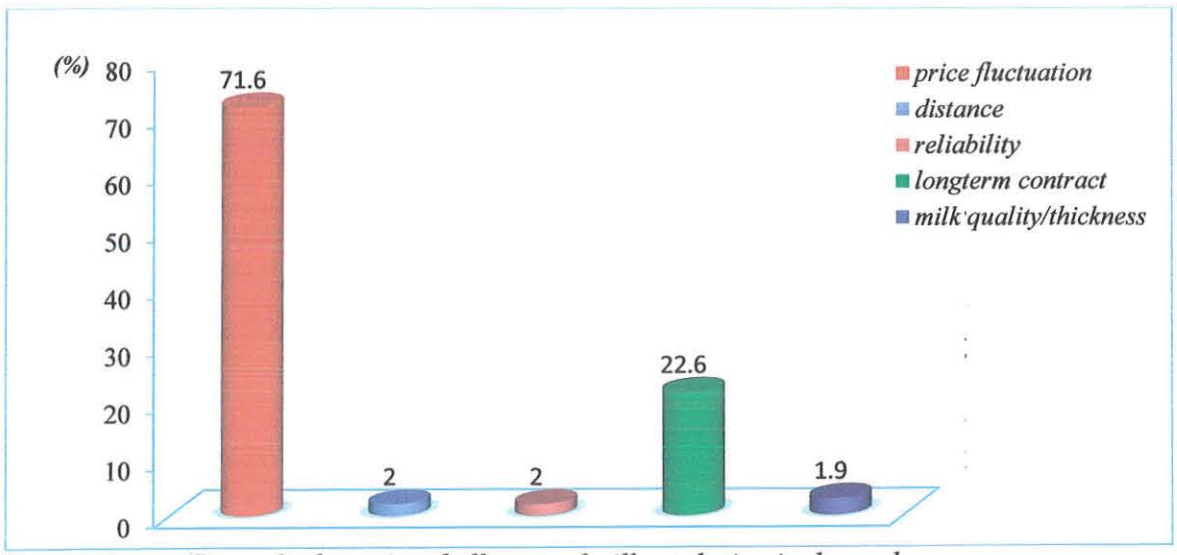


Figure 2: the major challenges of milk marketing in the study areas

As majority of community members in the areas were Orthodox Christian followers and they do have long fasting season that abstains consumption of animal products. This also resulted in price fluctuation in milk marketing. About 96% of the participants in the area responded that fasting season has a profound effect in the amount of milk marketed and diminution of its price. Besides to that, lack of well sophisticated transportation system, lack of consistent/long term customer flow especially during fasting season, lack of cooling system and lack of standard for pricing system have also their negative contribution to marketing of milk in the areas. Problems identified were slightly similar to that reported by (Teshager *et al.*,2013).

Majority of the participants in the study area complains that during fasting season both collection centers and private milk processing plants restricts the amount of milk to be brought to the center. These factors coupled with unavailability and expensiveness of raw materials in the area discourages milk producing households.

4.1.8 Awareness on milk production, transportation and marketing system

The level of awareness among producers play great role to maintain products in safer condition and good marketability of the products there by ensuring household food security as well improving economic status. However, although the sites are potential for milk production, majority of the participants were not in position to get support from responsible bodies for future expansion of the business and they have not got adequate training on milk production, transportation and marketing system.

According to the participants, only 52.5% of the respondents got training on milk production only from government where as the others were not well oriented in producing the product that penetrate the market and competitive in area.

The level of awareness contributes a lion's share in producing market competitive product there by maintaining household food security and national economy as well. Besides to this, awareness trigger producers to produce safe and quality item there by helps to reduce loss of product during milking, transportation and marketing chain. Due to lack of awareness, majority of the participants were not member of milk cooperative in the area.

Only 45.1% of the participants were members of milk cooperatives and others were not cooperative members that challenges them in marketing the products especially during fasting season. As majority of the participants said that those who are member of dairy cooperative were not face problem of milk marketing even during long fasting season because they have agreement in milk marketing throughout production period.

Use of detergent for cleaning and traditional flavoring plants for milking and milk storing equipments have significant effect on the microbial growth on the milk. However, almost all of the participants were using 'Ajekis' for washing milking and milk transporting equipments. Only insignificant numbers of the participants were use traditional flavoring plants for washing and smoking of milking, milk storage and transporting equipments.

4.2 Chemical quality of raw cow milk at different critical points

The major components of milk are water (87.4%), milk solids (12.60%), solids-not-fat (9.0%), fat (3.60%), protein (3.40%), milk sugar or lactose (4.90%) and ash or minerals (0.70%). These constituents may vary with genetic (breed and individual cow and variability among cows within a breed) and environment (interval between milking, stage of lactation, age, feeding regime, disease and completeness of milking)(Ramesh, 2006).

The raw cow milk at different points was subjected to a determination of the amount of total protein, fat and ash content and concentration of mineral elements. In addition the total solids (TS) and SNF was also analyzed and presented in Table 5.

The ash content which reflects the mineral composition of milk sample and ranged from 0.47±0.032 to 0.86%±0.067 and the milk sample collected from Sebeta collection center have the highest ash content and the least was from milk sample collected from Sululta retail shop.

The mean ash content obtained from present finding was in the range of Ramesh (2006) who reported 0.70%; Tola (2007) 0.70±0.01; Enb *et al.*(2009) 0.65 in raw cow milk in Egypt; but it was higher than that of Ibrahim *et al.* (2014); which was 0.37 from raw milk collected from different points in Nigeria.

The ash content of present study was showed a significant difference among critical points and between districts. Samples collected from Sebeta site had showed higher ash content than other sites.

The sample collected from Sebeta collection center had showed significantly higher($P<0.05$) ash content than other critical points. The variation in ash content of milk might be related to status of provision of mineral lick and supplements in the area and other environmental and genetic factors.

The protein content of milk sample ranged from 3.12 to 2.39, 3.29 to 2.70 and 3.04 to 3.08 at farmer and retail level of Sebeta, Holeta and Sululta, respectively. Sample collected from Sululta site had showed the highest protein content than other sites. Due to sample from Sululta collection center critical point had showed significantly higher($p<0.05$) protein content than other critical points. However, milk sample collected from Holeta retail shop had showed the least protein content.

Protein content of the present study was lower than that reported by Negash *et al.* (2012) which was 3.46 ± 0.04; but the present finding agrees with that of Rehrahie and Yohannes (2000) which was 2.67% protein; Ramesh (2006) 3.40% and Tola (2007), 3.31 ± 0.01.

The result was also within the range of the acceptable limit of cow milk protein of 2.9 to 5.0% that was reported by (O'Connor, 1994). The protein content of sample collected from Sebeta farmer had showed significant difference ($P<0.05$) than retail shop critical point.

However, the low protein content that decreasing along critical points from producer to final retail might be due to higher microbial load along the chain, lack of protein supplement for the animal and other environmental and genetic related factor of the animal.

The fat content of milk sample ranged from 4.47 to 3.76, 4.05 to 3.24 and 3.58 to 4.37 from farmer and retail shop of Sebeta, Holeta and Sululta, respectively. Due to the sample collected from Sebebta farmer had showed the higher fat content than other critical points and the least with milk samples collected from Sululta collection center. However, Sample collected from Sebeta informal merchants had showed significantly higher ($p<0.05$) fat content than other critical points.

There was significant difference in fat content of sample ($p<0.05$) among critical points and between districts. The result of proximate composition of raw cow milk from different critical points in the study sites was revealed in Table 5.

Table5: (Mean±SD)Percentage proximate composition of raw cow milk in different critical point at study sites

Districts	*Sample Source*	*Ash fb(%)*	*Protein (%)*	*Fat (%)*	*Total*	*SNF(%)*
Sebeta	SEF	0.60 ± 0.032^{a}	3.12 ± 0.133^{a}	4.47 ± 0.096^{a}	13.54 ± 0.29^{a}	13.02 ± 0.27^{a}
	SEC	0.86 ± 0.067^{b}	2.72 ± 0.190^{b}	3.25 ± 0.214	12.38 ± 0.46^{b}	12.38 ± 0.46^{b}
	SEIM	0.67 ± 0.020^{a}	2.59 ± 0.008^{b}	4.58 ± 0.085^{a}	13.65 ± 0.03^{a}	13.01 ± 0.04^{a}
	SERS	0.78 ± 0.002^{b}	2.39 ± 0.000^{c}		13.21 ± 0.02^{a}	12.64 ± 0.03^{b}
Holeta	HF	0.77 ± 0.025^{a}	3.29 ± 0.020^{a}	4.05 ± 0.083^{a}	10.86 ± 0.08^{a}	10.23 ± 0.90^{a}
	HOC	0.69 ± 0.042^{a}	3.27 ± 0.040^{a}	3.31 ± 0.118^{b}	11.38 ± 0.21^{a}	10.64 ± 0.20^{a}
	HIM	0.68 ± 0.142^{b}	2.28 ± 0.007^{b}	2.88 ± 0.298^{b}	10.38 ± 0.57^{b}	9.75 ± 0.59^{b}
	HDU	0.61 ± 0.033^{a}	2.79 ± 0.133^{b}	4.20 ± 0.151^{a}	10.81 ± 2.45^{c}	10.29 ± 2.44^{a}
	HRS	0.59 ± 0.247^{a}	2.70 ± 0.040^{b}	3.24 ± 0.222^{b}	11.67 ± 0.35^{a}	10.95 ± 0.35^{c}
Sululta	SUF	0.50 ± 0.007^{a}	3.04 ± 0.012^{a}	3.58 ± 0.028^{a}	10.54 ± 0.10^{a}	9.86 ± 0.10^{a}
	SUC	0.55 ± 0.065^{a}	3.41 ± 0.165^{b}	2.79 ± 0.016^{b}	9.82 ± 0.52^{a}	9.13 ± 0.52^{b}
	SUIM	0.49 ± 0.017^{a}	2.78 ± 0.253^{c}	3.10 ± 0.122^{c}	10.11 ± 1.18^{a}	9.65 ± 1.27^{a}
	SUDU	0.64 ± 0.095^{b}	2.92 ± 0.020^{c}	3.23 ± 0.099^{c}	10.04 ± 0.00^{a}	9.13 ± 0.00^{b}
	SURS	0.47 ± 0.032^{a}	3.08 ± 0.114^{b}	4.37 ± 0.137^{c}	11.85 ± 0.44^{b}	11.18 ± 0.43^{c}

The values were means of duplicate determinations. Means followed by different superscript letters for specific district within a column are significantly different($p<0.05$). SEF=Sebeta farmer, HF=Holeta farmer, SUF=Sululta farmer, SEC= Sebeta collection center, HOC= Holeta collection center, SUC=Sululta collection center, SUIM= Sululta informal merchants, HIM=Holeta informal merchant, SEM=Sebeta informal merchant, SUDU, Sululta dairy cooperative union, HDU=Holeta dairy cooperative union, HRS=Holeta retail shop, SURS=Sululta retail shop, SERS=Sebeta retail shop, FB=fresh weight base.

Besides, fat content of present study was in line with Ramesh (2006), 3.60%. On top of these, fat content of present finding was in the acceptable range of fat that is 2.5-6.0% reported by (O'Connor, 1994).

Although, the fat content of the present study was lower than that reported by Negash *et al.*(2012) 5.48±0.19 from East Shoa Zone of Oromia; Tola (2007) 6.05±0.02 from East Wollega; Rehrahie and Yohannes (2000) obtained 5.88% and Zelalem *et al.* (2004) reported 5.43%.

However, the lower fat content of present finding might be due to the high microbial load along different critical points in the study sites together with differences nutrition and genetic factors.

The mean total solid content of present study revealed that it ranged from 9.8 to 13.5 in Sululta collection center and Sebeta farmers respectively, which was agrees with that of Ramesh (2006),12.60%; Ibrahim *et al.*(2014) who found total solid content 11.69% from raw cow milk in Nigeria and Enb *et al.*(2009), 12.1±1.80.

However, it was lower than that of Tola (2007) who reported 14.31±0.03 from East Wollega of Oromia. The sample collected from Sebeta site had showed higher total solid content than other sites. Due to sample from Sebeta informal merchant had showed significantly higher($p<0.05$) total solid content than other critical points. The higher total solid content in the case of Sebeta may due to milking cows were feeding brewery by-product from Meta brewery factory that act as supplement for milking cows in the area.

Besides to this, the SNF (Solid-Not- Fat) content of milk samples ranged from 9.13±0.00 to 13.02±0.27 from Sululta dairy cooperative union and Sebeta farmers respectively. The lower SNF content was might be due to the higher moisture content reported from respective samples. The result of present study for SNF was higher than that reported by Tola (2007), 8.22±0.01; Zelalem *et al.* (2004), 8.43%; and Negash *et al.* (2012), 9.10 ± 0.09 from East Shoa and West Arsi Zones of Oromia.

However, the result for SNF of present study finding was agrees with the report of Rehrahie and Yohannes (2000), 9.27%. Negash *et al.* (2012) from East Shoa and West Arsi Zones of Oromia reported 5.48 ± 0.19, 9.10 ± 0.09 and 3.46 ± 0.04, for fat, SNF and protein respectively from raw cow milk collected from individual households. Similarity, Rehrahie and Yohannes (2000) obtained 5.88% fat and 9.27% SNF, but a slightly higher than 2.67% protein. The other study by Alganesh *et al.* (2007) reported 6.05% fat, 3.31% protein and 8.22% SNF.

On top of this, Zelalem *et al.* (2004) reported SNF, fat and protein percents, indicating 8.43, 5.43 and 3.17%, respectively from Ethiopian Boran cows.

The acceptable range of fat and protein from cow milk reported by O'Connor was between 2.5 to 6.0% and between 2.9 to 5.0% for fat and protein respectively (O'Connor, 1994).

4.3. Analysis of Mineral elements

Mineral elements are the most important inorganic component of food-stuffs that play a crucial role in many chemical reaction and biochemical process in the body. Calcium and phosphorous are required for bone formation both in infant and adult, for cell membrane permeability, for blood coagulation and muscle response. Zn is essential for basic physiological processes, development, lipid metabolism, brain and immune functions. Iron on the other hand is an integral parts of many proteins and enzymes that maintain good health. It is an essential component of protein and is involved in oxygen transport in the body.

Most of the trace elements are also present in milk at minute levels (Zinc, copper, iron, iodine, fluorine and selenium) and they perform several vital body functions as catalyst, activators and regulators (IDF, 2008). According the report of USDA (2008), the mineral elements specially calcium, phosphorus and iron in raw cow milk in mg/100gm were 113, 91 and 0.03 respectively.

Dawd *et al.* (2012) reported average concentrations of the mineral element Zn and Fe were (4.923±0.277mg/kg),(1.213±0.077mg/kg) respectively for raw cow milk samples collected from selected sub-cities in Addis Ababa. Ghada (2005) reported the main mineral elements from raw cow milk in Egypt; Ca, P, Zn and Fe 119±0.690, 95.03±0.72, 0.38±0.00 and 0.070±0.02mg/100g respectively. The result for mineral element of milk samples are shown in the Table 6.

Table 6: Mean±SD values of mineral elements in raw cow milk in different critical points at study sites(*mg/100g*)

District	*Source of Sample*	*Ca*	*Fe*	*Zn*	*P*
Sebeta	SEF	124.69±1.23[a]	0.068±0.00[a]	0.299±0.00[a]	89.850±1.41[a]
	SEC	122.34±2.27[a]	0.069±0.01[a]	0.299±0.01[a]	89.141±0.68[a]
	SEIM	119.93±4.00[b]	0.071±0.01[b]	0.310±0.09[a]	87.224±0.45[b]
	SERS	123.49±0.24[a]	0.071±0.00[b]	0.306±0.09[a]	88.051±0.19[b]
Holeta	HF	116.31±1.60[a]	0.082±0.02[a]	0.356±0.03[a]	92.474±1.58[a]
	HDU	121.07±6.90[b]	0.086±0.00[b]	0.350±0.02[a]	93.014±0.98[a]
	HRS	120.64±4.57[a]	0.060±0.00[c]	0.346±0.03[b]	92.236±1.13[a]
Sululta	SUF	116.18±1.10[a]	0.072±0.01[a]	0.348±0.04[a]	91.707±0.75[a]
	SUC	117.23±1.58[a]	0.074±0.01[a]	0.347±0.08[a]	90.562±0.98[a]
	SUIM	118.01±1.67[a]	0.062±0.00[b]	0.354±0.03[b]	91.077±1.70[a]
	SUDU	117.80±2.78[a]	0.069±0.00[a]	0.355±0.00[b]	90.819±1.99[a]
	SULRS	117.87±0.27[a]	0.072±0.01[a]	0.353±0.08[b]	89.951±1.62[a]

The values were means of duplicate determinations. Means followed by different superscript letters for specific district within a column are significantly different($p<0.05$). SEF=Sebeta farmer, HF=Holeta farmer, SUF=Sululta farmer, SEC= Sebeta collection center, HCC= Holeta collection center, SUC=Sululta collection center, SUIM= Sululta informal merchants, HIM=Holeta informal merchant, SEM=Sebeta informal merchant, SUDU, Sululta dairy cooperative union/Selale, HDU=Holeta dairy cooperative union, HRS=Holeta retail shop, SURS=Sululta retail shop, SERS=Sebeta retail shop.

The mean values for mineral element Ca in present study varied from 124.7, 116.3, 116.18 and 123.49, 120.64, 117.87mg/100g from farmer and retail at Sebeta, Holeta and Sululta, respectively. The results of present finding were higher than USDA (2008) report, calcium in mg/100gm in raw cow milk was 113; but it was in line with that of Ghada (2005) reported Ca,119±0.690mg/100gm.

Sample collected from Sebeta retail shop had showed higher Ca content than other critical points. Due to sample collected from critical point Farmer had showed significantly higher($p<0.05$) Ca content than other critical points. There was significant difference($P<0.05$) in Ca content between critical point farmer and informal merchants of Sebeta; besides, value obtained from Sebeta site was significantly higher($P<0.05$) than Sululta sites. However, Ca content of sample collected from Sebeta was not significantly different ($p<0.05$) from that of Holeta except for sample from farmers.

The mean values of Zn ranged from 0.299 to 0.356, 0.348 to 0.306 and 0.346 to 0.353 for Sebeta, Holeta and Suluta farmers and retail shops, respectively. Sample from Sululta site had showed higher Zn content than Sebeta site. However, sample collected from Holeta farmer had showed significantly higher ($p<0.05$) than other critical points. Besides, sample collected from Sululta site was significantly higher($p<0.05$) than Sebeta site. However, the results of present finding was lower than that of Ghada (2005), who reported mineral element Zn 0.38±0.00mg/100gm from raw cow milk in Egypt.

But it was higher than that of Dawd *et al.* (2012) who reported average concentrations of the mineral element Zn (4.923±0.277mg/kg) in selected sub-cities in Addis Ababa. There was a significant difference($P<0.05$) observed in the Zn content among critical points of present study except in the case of Sebeta.

The mean value of mineral element Fe ranged from 0.068 to 0.071, 0.082 to 0.060 and 0.072 to 0.072 mg/100gm for samples collected from Sebeta, Holeta and Sululta farmers and retail, respectively. The values of present study was higher than that of Ghada (2005) but it was lower than that of Dawd *et al.* (2012) and USDA (2008) report which was 1.213±0.077mg/kg and 0.03mg/100gm respectively. Higher Fe content was obtained from samples collected from Holeta farmers.

However, sample collected from Holeta dairy Cooperative Union had showed significantly higher($p<0.05$) Fe content than other critical points. Besides, sample from Holeta farmer had showed significantly higher($p<0.05$) Fe content than Sebeta and Sululta farmers. The Fe content was significantly ($p<0.05$) different between critical points of the study areas.

The mean values of Phosphorus from present study was varied 89.850 to 88.051, 92.474 to 92.236 and 91.707 to 89.951 at Sebeta, Holeta and Sululta farmers and retail shop, respectively. Samples collected from Holeta dairy cooperative Union had showed higher P content than other critical points of the study sites.

However, the values of present study was in line with that of USDA (2008) report which was 91gm/100gm; but it was lower than that reported by Ghada (2005), 95.03±0.72 mg/100g.

Samples collected from Holeta sites had showed significantly higher($p<0.05$) P content than Sebeta sites and there was significant difference($p < 0.0.5$) in phosphorus content within critical points and between districts.

The significant difference of mineral element between districts might be due to differences in topography of the area, type of feed provided for milking cows, blood level of milking cows, lactation stage and level of mineral supplements provided to the dairy cows.

4.4 Microbial Analysis of raw cow milk

The microbial quality of milk indicates the hygienic levels during milking that include cleanliness of the milking utensils, proper storage and transport as well as the hygienic status of the udder of the individual cow (Spreer, 1998). Standard plate count (SPC) is one of the most commonly used microbial quality tests for milk and milk products.

The total aerobic bacterial counts (TABC) obtained from farmer level raw milk sample ranged from $4.78x10^4$ to $8.29x10^6$log cfu/ml with an average value of 6.88±0.46log *cfu/ml* and the total aerobic bacterial count of milk samples obtained from dairy cooperative union and retail shop ranged from $3.85x10^2$ to $7.79\ x10^6$ log10*cfu/ml at* Sululta to $6.86x10^5$ to $7.88x10^6$ at Holeta, respectively and $7.55x10^5$ to $8.49x10^7$ and $7.14x10^5$ to $7.26\ x10^5$ log *cfu/ml* at Holeta and Sululta retail shops respectively. However, lower total aerobic bacterial count was obtained from Sebeta retail shop with the mean± SD of 6.7±0.694log *cfu/ml*. On other hand, the mean value of total aerobic bacterial count obtained from informal merchant at Sululta and Holeta was 8.07±0.834log *cfu/ml* and 7.45±0.264 log *cfu/ml*.

The value of total aerobic bacterial count for present study revealed lower than that reported by Tola (2002) in Eastern Wollega that had average count 7.4x10; Beyene (1994) in Southern Ethiopia that had average count of 7.7log *cfu*/ml; Tassew & Seifu (2011) at Bahir Dar Zuria with the overall mean of 7.58log10*cfu*/ml; Worku *et al.*(2012) who reported bacterial count from 7.36 -7.88 log10 *cfu*/ml of raw cows' milk in Borana, Ethiopia and Mosu *et al.* (2013) at selected dairy farms in Debre Zeit town that had the average total bacterial count of 7.07log *cfu*/ml.

However, the mean total bacterial count of milk samples obtained from present study was higher than Tesfay *et al.* (2013) at Dire Dawa town with mean total bacterial count of 5.84±0.629 *cfu*/ml. On the other hands, mean values of total bacterial counts obtained from informal merchants and retail shops were higher than that reported by Tesfay *et al.* (2013) with mean value of 9.137±0.885*cfu*/ml. The total aerobic bacterial count obtained from retail shop were significantly higher ($p<0.05$)than milk samples collected from households/farmers.

The higher total aerobic bacterial count observed in the present study may be attributed to the initial contamination of milk samples either from of the cow, milkers hand, milking areas and container itself. On the other hand, high bacteria count observed in milk samples collected from informal merchant and retail shop could probably be due to further contamination of the milk during transportation, extremely high transportation temperature, the use of poorly cleaned milk containers, lack of and improper cooling systems at milk vending areas and poor personnel hygiene.

The higher count indicates substandard hygienic conditions practiced during milking and subsequent handling. This implies that the sanitary conditions in which milk has been produced and handled are substandard subjecting the product to microbial contamination and multiplication. It is indicated that total bacterial count is a good indicator for monitoring the sanitary conditions practiced during production, collection, and handling of raw milk (Fatine *et al.*, 2012).

Hence training of milk handlers about hygiene can significantly reduce the bacterial load in milk. A good example for this could be reduced total bacterial count observed in milk sampled from farmers who received training on hygienic milk production and handling (Nebiyu, 2008; Sintayehu *et al.*, 2008).

Milk produced under hygienic conditions from healthy cows should not contain more than 4.7 log10 *cfu/ml* (O' Connor, 1994). Table 7 below indicates aerobic bacterial counts in different critical points and study district.

Table 7: Mean (± SD) Aerobic mesophilic bacteria counts of raw milk samples (log10 *cfu/ml*) collected from different Value chain/critical points of the study sites.

	Study districts				
Sample sources	***Sebeta***	***Holeta***	***Sululta***	***Standard***	***Authority***
Farmer	6.48±0.065[a]	7.2±1.152[a]	7.02±0.169[a]	10^5	ICMSF[2000]
Collection center	6.80±0.031[b]	7.64±0.034[b]	7.87±0.490[b]	$2*10^5$	WHO[1981]
Informal merchant	6.89±0.178[b]	7.45±0.264[a]	8.07±0.834[b]	$<10^5$	APHA [1992]
Milk cooperative	-	6.10±1.086[b]	5.96±1.160[c]	$5*10^4$	ECC [2001]
Retail shops	6.7±0.694[b]	7.88±0.416[c]	7.20±0.056[a]		

The results were from duplicate values. Mean±SD values indicated by different superscript with in a column have significant difference at ($p<0.05$).

The present result revealed that there is increment of bacterial count at each critical points. The mean (± SD) bacterial count was 6.48±0.065, 7.2±1.152and 7.02±0.869 log10 *cfu/ml* in dairy farmers, 6.7±0.694, 7.88±0.416 and 7.20±0.056 log10 *cfu/ml* in milk vending/retail shops of Sebeta, Holeta and Sululta, respectively. This could be due to improper handling, storage and transport time after the milk leaves the dairy farms. There is a significant difference in the total aerobic bacterial counts in different critical points in the study areas and between districts at ($p<0.05$).

Coliform counts can indicate fecal contamination or contamination from equipment that has not been properly cleaned and sanitized (Schmidt, 2008; Bintsis *et al.*,2008; Biruk *et al.*, 2009). As indicated in Table 8, the overall mean (± SD) of feacal coli form counts of present study at farmer level ranged from 5.42±1.7352, 5.53±1.0345, 4.18±1.2286 *log10cfu*/ml at Sebeta, Holeta and Sululta, respectively.

The coli form count obtained in the current study is higher than Tassew and Seifu (2011) at Bahir Dar Zuria with the mean value of 4.49 log *cfu/ml*; Fekadu (1994) who found coli form counts of 3.8, 4.0 and 3.8log10cfu/ml for cow milk produced in Aneno, Gulgula and Dongora districts of Southern region respectively; Worku *et al.* (2012) found overall coli form counts of 6.88±0.040 and 7.786.88±0.040 at cow udder and storage containers respectively in Borana pastoral community of Oromia region; Tesfay *et al.* (2013) with the mean value of 4.13 ± 0.757 log10 *cfu/ml* for milk samples collected from dairy farms at Dire Dawa town; Tola (2002) raw cow's milk sampled from smallholder producers contained coli form counts of about 4.46 log *cfu*/ml;

but it was lower than Zelalem and Faye(2006) who reported higher coli form count of 6.57*cfu/ml* for cow milk collected from different producers in central highlands of Ethiopia. On the other hand, the mean coli form counts obtained from retail shops of present study was higher than the above research works.

Table 8: Mean (± S.D) value of Coli form counts of raw milk samples (log10 *cfu/ml*) collected from different sampling points of the study areas.

Source of Sample	*Study districts*				
	Sebeta	*Holeta*	*Sululta*	*Standard*	*Authority*
Farmer	5.42 ± 1.735^{a}	5.53 ± 1.034^{a}	4.18 ± 1.228^{a}	10^3	ICMSF[2000]
Collection center	5.44 ± 0.979^{a}	5.92 ± 0.620^{a}	7.13 ± 0.305^{b}	10^2	WHO[1981]
Informal	5.47 ± 1.462^{ab}	6.10 ± 0.917^{b}	6.38 ± 0.616^{c}	$<10^2$	APHA [1992]
Milk cooperative	-	7.68 ± 0.509^{c}	6.15 ± 0.913^{c}		
Retail shops	5.78 ± 0.985^{b}	5.63 ± 0.625^{a}	6.35 ± 0.435^{c}		

The results were from duplicate values. Mean±SD values indicated by different Superscript within the same column were significantly different at ($p<0.05$). standards were for raw milk available for direct human consumption.

The mean coli form counts was ranged from 5.42±1.735 to 5.78±0.985 for sample collected from farmer and retail shop of Sebeta site. Sample from critical point retail shop had showed significantly higher ($P<0.05$) coli form count than farmer sample. Besides, sample collected from Sululta retail shop had showed significantly higher counts than farmer level sample. Coli form counts had showed significant difference among critical points and between districts. This might be due to cross contamination of milk along different critical points in chain and initial feacal contamination of the sample together with poor handling during transportation and storage.

The presence of *E. coli* organisms in milk and milk products is an indication of unsanitary production and\or improper handling of either milk or milk utensils (El-zubeir and Ahmed, 2007 & Olfa et *al.*, 2013). Milking udder with sub-clinical mastitis and wet environment lead to contamination of bulk tank milk and hence raw milk reaches the consumers with elevated Coliform count (FAO,2008; Zadoks *et al.*, 2007).

Unclean hands of workers, contaminated milk, unhygienic conditions of the manufacturing unit and water supplied for washing the utensils could be the source for accelerating the bacterial contamination of milk products beside the post manufacturing contamination (Elmahmood *et al.*, 2007).

Recovery and counting of *E. coli* is used as reliable indicator of fecal contamination and a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard (Kaper *et al.*,2004). The values of E.coli counts of present study from critical points was presented in table 9

Table 9: Mean (± S.D) *E. coli* counts of raw milk samples (Log10 cfu/ml) collected from different critical points of the study sites.

Sample sources	***Study districts***				
	Sebeta	Holeta	Sululta	Standard	Authority
Farmer	3.19 ± 1.704^{a}	1.53 ± 0.007^{a}	1.19 ± 0.266^{a}	$1*10^{5}$	FDA[2001]
Collection center	3.30 ± 0.421^{a}	1.61 ± 0.427^{a}	2.29 ± 1.285^{b}	$0*10^{1}$	ICMSF[2000]
Informal merchants	-	2.56 ± 1.961^{b}	2.16 ± 0.066^{b}		
Milk Coop. Union	-	1.90 ± 1.540^{c}	2.86 ± 1.807^{c}		
Retail shops	$4.17\pm^{b}$	1.53 ± 0.141^{a}	1.76 ± 0.033^{d}		

The results were from duplicate values. Mean±SD values indicated by different Superscript within the same column were significantly different at ($p<0.05$).

The finding of the present study indicated that milk samples collected from different critical points in the study sites were highly contaminated with *E.coli.* From the total of 60 samples collected from different critical points in the study site; *E.coli* was isolated from 26(43.33%) of the samples with varying levels; 26.92, 7.96, 3.84, 3.84, 3.84, 3.84, 7.69, 7.69, 11.53, 11.53, and 11.53% were isolated from Sululta dairy cooperative, Sululta informal merchant, Sebeta retail hop, Sululta retail shop, Holeta farmer, Sululta farmer, Holeta dairy union, Sebeta farmer, Holeta informal merchant, Holeta retail shop and Sululta Collection center, respectively.

The result of present study is in line with Endale *et al.* (2013) at different critical points in Mekelle (44.4%);11.1% at farm level, 11.1% at milk vending shops, 22.2% at cafteria and Vahedi *et al.* (2013) in 42 (42%) from raw cow milk; but it is higher than that reported by Olfa *et al.* (2013) 13 out of 50 milk samples (32.5%) were contaminated with *E. coli* in Sfax, Tunisia from raw cow's milk from different localities.

The mean value of *E.coli* from present study from Sebeta retail shop is higher than Tesfay *et al.* (2013) who reported *E. coli* count of raw milk samples collected from dairy farms were 3.64 ± 0.776 *cfu/ml* at Dire Dawa; but it is lower than his report at other critical point.

In contrast to this, the value of *E. coli* from present study is lower than the reported value for *E.coli* (3.93 ± *0.01cfu/ml*) by Ali and Abdelgadir (2011) from raw milk samples. However, samples collected from dairy cooperative union in the present study had implicated higher *E. coli* counts than other critical points. It indicates that there is increment in microbial load along different critical points of milk marketing from farmer to the consumer level.

This may be due to cross contamination of milk during transportation, lack of sanitation of storage container and lack of temperature control through the chain that create conducive environment for multiplication of particular microorganism. *E. coli* count in milk samples obtained from retail shop was significantly higher ($p<0.05$) than milk samples obtained from dairy farmer for Sebeta site.

Milk and dairy products are frequently contaminated with enterotoxigenic *Staphylococcus* species, which are often involved in SFP, especially in areas characterized by a high level of consumption of these products, since staphylococci are often involved in cases of subclinical mastitis of ruminants resulting in contamination of milk (Salandra *et al.*, 2008). Raw milk is a potential source of staphylococci, especially in the case of mastitic milk and defective pasteurization (Kaloreu *et al.*, 2007).

The findings of present study revealed that *Staphylococcus aureus* was isolated from 17(28.33%) of samples collected from different critical points in the study sites. The findings of the present study was in line with that of Endale *et al. (2013)* at Mekelle *Staphylococcus aureus* was isolated from 48 samples (26.7%),milk samples collected from dairy farms and vendors from Mekelle.

However, it is higher than that reported by Mekuria *et al.* (2013) reported prevalence of 51 (15.5%) *S. aureus* out of the total samples examined from selected dairy farms around Addis Ababa; Aydin *et al.* (2011), reported10.2% of *S.aureus* in raw milk samples collected from Turkey and Vadehi *et al.* (2013) 22(22%) of *S.aureus* in the raw milk samples from farms. But present finding was lower than that of Mekonnen (2009) who reported the prevalence of *Staphylococcus* 33% and 46% from buckets milk and tanks milk from Debre Zeit, respectively; Hempen *et al.* (2004) from Gambia, reported 33.3% of the

raw milk samples showed counts of coagulase-positive *Staphylococci spp.* above $2x10^3$*cfu*/ml.

The findings of the present study may be due to lack of hygienic bedding condition as reported from majority of the study participants which is predisposing factor for mastitis that is complex of soiling of udder that favors further contamination and growth of bacteria, lack of washing udder and teat before and after milking, occurrence of sub-clinical mastitis and lack of overall hygienic condition during milking, storage and transportation.

Salmonella is an enteric bacteria and is the most common food-borne pathogen (Weigel *et al.*, 2004 and Mizumoto *et al.,* 2005). *Salmonella* are mostly facultative anaerobes, oxidase-negative, catalase-positive and gram negative rods. Most strains are motile and ferment glucose with production of both acid and gas. *Salmonella* have several sub species. *Salmonella enterica* is the most responsible for 99.9% infection in humans and most of infection are zoonotic in origin (Yan *et al.*, 2003).

According to Jayaroo and Henning (2001) *Salmonella* was isolated from 6.1% of bulk tank milk sample from dairy herds in South Eastern Dakata and Western Minnesota. In other study conducted in Addis Ababa salmonella was isolated from 2.1% of milk samples collected from different supper market in Addis Ababa (Tesfaw *et al.*, 2013). Tesfay *et al.* (2013) reported raw milk samples were positive for *Salmonella* spp. with a percentage of detection of 18.8% and 41.7% for milk samples obtained from dairy farms and vendors, respectively from Dire Dawa; Van Kassel *et al.* (2004) reported a 2.6% occurrence of *Salmonella* spp. in raw milk samples collected from US dairies. However, *Salmonella* was not detected in the present study.

In the findings of yeast and mold of present study, highly varied from farmer to retail levels. However, the values of present findings were lower than that reported by Ahmed (2011), who reported 6.1 and 7.4log *cfu/ml* for yeast and mold for raw camel milk from Mieso districts of Oromia region. It was higher than that Karmen and Slavica (2008) and Fadda *et al.* (2004) who reported 2.3 *log cfu/ml* and 2.64 *log cfu/ml,* respectively.

The findings of yeast and mold was highly varied from farmer to retail levels and showed in the table below(Table 10).

Table 10: Mean (± S.D) Mold and Yeast counts of raw milk samples (Log10 *cfu/ml*) collected from different critical points of the study sites.

Sample Source	*Districts*					
	Sebeta		*Holeta*		*Sululta*	
	yeast	*mold*	*yeast*	*mold*	*yeast*	*mold*
Farmer	3.77 ± 0.47^{a}	3.45 ± 0.26^{a}	2.46 ± 1.15^{a}	2.30 ± 0.19^{a}	2.16 ± 1.25^{a}	2.99 ± 0.82^{b}
CC	3.76 ± 0.44^{a}	3.46 ± 0.08^{a}	3.24 ± 0.46^{a}	3.26 ± 0.04^{b}	3.16 ± 0.91^{b}	3.79 ± 0.70^{c}
IM	3.76 ± 0.41^{a}	3.51 ± 0.10^{a}	3.73 ± 0.42^{b}	2.43 ± 0.17^{a}	3.78 ± 0.10^{c}	4.72 ± 1.16^{d}
DCU	-	-	3.55 ± 0.52^{c}	3.45 ± 0.26^{c}	4.16 ± 0.34^{c}	3.73 ± 1.10^{c}
RS	3.85 ± 0.42^{b}	3.82 ± 0.76^{b}	3.59 ± 1.44^{c}	3.38 ± 0.48^{c}	2.26 ± 1.07^{a}	2.41 ± 0.15^{a}

The results were from duplicate values. Mean±SD values indicated by different Superscript within the same column were significantly different at ($p<0.05$).CC=collection center, IM=informal merchant, DCU=Dairy Cooperative Union and RS=Retail shop

The mean±SD of yeast counts varied between 3.77±0.47 and 3.85±0.42 for samples collected from Sebeta district(Table 10). Samples due to critical point retail shop was significantly higher than other points ($p<0.05$), mean±SD of mold counts were varied between 3.45±0.26 and 3.82±0.76 for the sample Collected from Sebeta district. The mean±SD of yeast counts were varied between 2.46±1.15 and 3.59±1.44 for the sample collected from Holeta district. Samples due to critical point informal merchant was significantly higher than other critical points($P<0.05$). Mean±SD counts of mold counts were varied between 2.30±0.19 and 3.38±0.48 for samples collected from Holeta district. Samples collected from retail shop was significantly higher than all other critical points($p<0.05$).

However, their values increased significantly at retail shops following the chain in the respective districts except for retail level of Sululta. The value for mold count was varied from 3.45 to 3.82, 2.30 to 3.38 and 2.99 to 2.41 at farmer and retail level of Sebeta, Holeta and Sululta, respectively. The value for yeast count was varied from 3.77 to 3.85, 2.46 to 3.57 and 2.16 to 2.26 at farmer and retail level of Sebeta, Holeta and Sululta, respectively. Due to sample collected from retail level of Sebeta and Holeta had showed significantly higher ($p<0.05$) yeast and mold counts than farmer level. However, the value for yeast in the case of Sululta was not significantly different between farmer and retail level.

This might be due to location of the areas; high altitude together with high relative humidity that favors the growth of molds, feeding stored feed that developed molds, lack of hygienic practices especially washing milking and milk storing utensils, mixing of cold and newly drawn milk and storing together.

5. Conclusion and Recommendation

5.1 Conclusion

Milk is one of the most important nutrients dense food, because it is an excellent source of essential nutrients and casein, and its consumption plays a significant role for good health. Milk is, however, a conducive medium for growth of pathogenic microorganisms due to its ideal pH and high moisture content. Milk production and marketing is one of the most important farm activities that helps to generate income for households, maintain household food security in study areas and contributes to national economy as well.

Milk production in the study sites was highly constrained by production, handling and marketing problems that reduce the amount to be produced, safety of the product and uniform distribution of particular food item between or within group/food security in particular. The major problems identified in the areas were feed shortage and its high cost as well as price fluctuation between fasting and no fasting periods of milk consumption.

Farm households market raw whole milk mainly to private milk processing plant, milk collection center and dairy cooperative unions rather than local market in the study area. The result obtained in this study concluded that milk available to the consumer in Addis Ababa via different supply chain critical points have a high bacterial load beyond acceptable critical limits according to American and European community member states. Also the milk considered from the study areas were contaminated with most hazardous agents such as *Staphylococcus species., E. coli,* yeast and mold. It indicates that hygienic procedures were not strictly followed during milk production to supply route.

Samples collected from Sebeta site had showed higher Calcium content than other study areas but lower Zinc and Phosphorus content. Whereas samples collected from Holeta site had showed higher Fe and Phosphorus content.

The nutritional composition of milk from different sampling points in the study sites were varied along the critical points and between districts. Generally, the study showed that the quality of milk obtained from the different sources such as dairy farmers, collection centers, informal merchants, dairy cooperative union and retail shops were substandard [compared to relevant North American or EU regulations].

5.2 Recommendations

The finding indicated standard sanitary operation practices should be applied at unit operations such as at the regular washing and sterilization of dairy equipment, utensils, milkers' hand, udders, isolation of diseased animals, and pasteurization of milk before collection and distribution of milk for consumption. The magnitude of the problem of bacterial contamination deserves more elaborative studies from the point of production of milk to the point of milk retail for consumption.

The results of the present study indicate that strict preventive measures should be adopted to ensure contamination free milk and its products for the good health of all consumers. Therefore, stakeholder authorities should regularly monitor the overall hygienic conditions of the milk production and conduct frequent inspections of milk marketed in Addis Ababa to check whether or not the minimum legal standards are met. Remedial actions can be taken by:

- Government, different NGO's and other responsible bodies should participate in training producer, milk collecting centers, merchants and retail shops to maintain the safety and quality of milk from farm to fork.
- It is imperative to develop and implement guidelines in milk marketing chain there by encourage consumption of safe product and minimize loss of product due to spoilage.
- The Government should create awareness on integrative farming system i.e. the NGO'S, milk processing plants, and dairy cooperative unions supply input for dairy farmer and farmer should sale the product by pair price to maintain safe and sustainable milk production in the area and national level as whole.
- Milk marketing actors especially from collection center to retail shop and/vendors should use refrigerated vehicle and cold chain in place of open container and vehicle to maintain bulk tank temperature there by minimize microbial growth during transportation and storage.
- Actors in each critical point should perform basic laboratory test for at least indicator microorganisms that are frequently detected in raw milk available for direct human consumption.

- Since there is long term fasting season that hinder consumption of milk and milk products continues, which results in low price and loss of products therefore, cottage milk processing industries should be mandatory to establish in the areas to maintain sustainable production of milk and its products in the areas.

References

Ackland, M., and Michalczyk, A., (2006). Zinc deficiency and its inherited disorders-A review. *Genes & Nutrition* 1: 41-50.

Adil, M. A, S and Iman M. H. (2011). 'Enumeration and identification of *Coliform* bacteria from raw milk in Khartoum State'. *Sudan Journal of Cell and Animal Biology* 5(7):121-128.

Agza B., Melesse K., Funga A. and Kassech M.(2013). 'Assessment of knowledge gap and factors affecting consumption of dairy products in Ada'a & Lume districts of East Showa Zone, Ethiopia.' *African Journal of Food Science and Technology* 4(9):201-210.

Ahmed, M. E. (2009). 'The association between bulk tank milk analysis for raw milk quality and on-farm manage. practices. URL:udini.proquest.com/view/the-association-between-bulk-tank-pqid:1855008811/. Accessed at 22/ 11/2013.

Ahmed, M.I.(2007).'The hygienic quality of raw milk produced by some dairy farms in Khartoum-Sudan'. *Journal of Microbiology*, 2: 988-991.

Ahmed, A.(2011). Investigation on nutritional and microbiological properties of camel *(camelus drodedarius)*milk :a case study of Mieso district, Oromia, Ethiopia MSc. Thesis Submitted to Center for Food Science and Nutrition, Addis Ababa University, Ethiopia.

Ali, AA., Abdelgadir, WS.(2011). 'Incidence of Escherichia coli in Raw Cow's Milk in Khartoum State'. *British Journal of Dairy Sciences* 2, 23-26.

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

AOAC. Official Method of Analysis AOAC, International (2005) 18th Ed. Determination of minerals in foods by Atomic Absorption Spectrophotometer method ,Association of Official Analytical Chemists, Gaitherburg, Maryland, USA.

APHA (1985). Chemical and Physical methods, In G. H. Richardson(ed), Standard methods for the Examination for Dairy products, 15th Ed. American Public Health Association, Washington, D.C., Pp 327-404.

APHA (1992). Standard Method for the Examination of Dairy Products. 16th ed., American Public Health Association (APHA), Washington.

Argudin, M.A., Mendoza, M.C., Rodicio, M. R (2010). 'Food poisoning and Staphylococcus aureus enterotoxins. Toxins 2(7):1751–1773.

Asperger, H., Zangerl P. (2003). *Staphylococcus aureus*. In: Roginski H, Fuquay J, Fox P, editors. Encyclopedia of Dairy Science. San Diego: Academic Press pp. 2563-2569.

Ashenaf, M. (2002). Microbiology of Ethiopian foods and beverages: A review. SENET'. Ethiopian journal of science; 25(1):97-140.

Aycicek, H., Cakiroglu, S. and Stevenson, T.(2005). Incidence of *Staphylococcus aureus* in ready to eat meals from military cafeterias in Ankara, Turkey. *Food Control,* 16: 531–534.

Aydin, A., Sudagidan M. and Muratoglu K.(2011). 'Prevalence of staphylococcal enterotoxins, toxin genes and genetic relatedness of food-borne *Staphylococcus aureus* strains isolated in the Marmara region of Turkey'. *International Journal of Food Microbiology* 148:99-106.

Azage, T.(2003). Financing market oriented dairy development: the case of Ada'a-Liben districts Dairy Association ,Ethiopia. Urban Agricultural Magazine. No.9. Koninklije.

Barbano, D M., Ma. Y, Santos, M. V.(2006). 'Influence of raw milk quality on fluid milk shelf life'. *Journal of Dairy Sci.* 89:15–19.

Belete Anteneh, Azage Tegegne, Fekadu Beyene and Berhanu Gebremedhin (2010). Cattle milk and meat production and marketing systems and opportunities for market-orientation in Fogera woreda, Amhara region, Ethiopia. IPMS (Improving Productivity and Market Success) of Ethiopian Farmers Project Working Paper 19. ILRI (International Livestock Research Institute), Nairobi, Kenya. 65 pp.

Benkerroum, N., Bouhal, Y., EI Attar, A. and Marhaben, A. (2004). 'Occurrence of Shiga toxin-producing *E. coli* 0157:H7 in selected diary and meat products marketed in the city of Rabat'. *Morocco Journal of Food Protection* (67)6:1234–1237.

Beyene F. (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. Ph.D. Thesis, Department of Food Science. Agricultural University of Norway. Norway.

Bintsis, T, Angelidis A S and Psoni, L. (2008). Modern Laboratory Practices: Analysis of Dairy Products. In: Advanced dairy science and technology. Britz, T. J. and Robinson R. K. (Eds.). Blackwell Publishing Ltd, UK.

Biruk, H, Samson, W, Zelalem, Y (2009). Microbial properties of milk and traditionally produced Ethiopian fermented milk products: a review. Proceedings of the 17th Annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, September 24 to 26. pp. 137-148.

Blowey RW.(1999). A Veterinary Book for Dairy Farmers. 3rd Edition. Farming Press. Ipswich. United Kingdom; pp186.

Canabady-Rochellea, L S, Mellemab, M.(2010); Physical- chemical comparison of cow's milk proteins versus soy proteins in their calcium-binding capacities. *Colloids and Surfaces A: Physicochem. Eng. Aspects 366*, 110- 112.

Cannas, J., Noordhuizen J. (2008). 'Consumer Safety and HACCP-Like Quality Risk Management Programs on Dairy Farms: The Role of Veterinarians'. *The Open Vet Science Journal*; 2:37-49.

Caswell, JA.(2003). Food safety. In L. J Unnevehr (Ed.), Food Safety in Food Security and Food Trade. Washington: International Food Policy Research Institute.

Chye, FY., Abdullah, A., and Ayob, MK. (2004). Bacteriological quality and safety of raw milk in Malaysia. Food Microbiol 21:535-41.

Coni, E, Bocca, B, Caroli, S. (1999). 'Minor and trace element content of two typical Italian sheep dairy products'. *Journal of Dairy Research 70*, 355-357.

Coorevits, A., De Jonghe, V., Vandroemme, J., Reekmans, R., Heyrman, J., Messens, W., De Vos, P., Heyndrickx, M.(2008). Comparative analysis of the diversity of aerobic-spore-forming bacteria in raw milk from organic and conventional dairy farms. System. *Applied Microbiology.*

CSA.(2004).The 20001/2002 Ethiopian Agricultural Sample Enumeration(EASE) Executive Summary. Central Statistical Authority, Addis Ababa, Ethiopia.

CSA.(2010). Federal Democratic Republic of Ethiopia Central Statistical Authority. Livestock and livestock characterization. Agri. Survey. 2(468): 107.

CSA (Central Statistical agency) (2012). Ethiopia Sample survey Enumeration. Addis Ababa Ethiopia.

CSA (2013). Agricultural Sample Survey 2012/13 [2005 E.C.] *Volume II.* Report on Livestock and Livestock Characteristics (Private Peasant Holdings) Federal Democratic Republic of Ethiopia Central Statistical Agency (CSA).Addis Ababa, Ethiopia.

Deutchen Forschungsanstalt für Lebensmittelchemie (2012); Food composition and nutrition tables. Medline online database, Leibniz Institut. http://www.sfk-online. net/cgi-bin/sfkstart.mysql?language=English (assessed on 03.04.2014.).

Dawd, AG., Gezmu TB., and Haki GD.(2012). 'Essential and toxic metals in cow's whole milk from selected sub-cities in Addis Ababa, Ethiopia.' *Online International Journal of Food Science*;1(1):12-19

Douglas JR (2003). Bulk tank cultures are the dairy man best friend. University of Wisconsin Milking Res. Inst. Lab., (Report) No. 2223.

El-Behoos St., Dokki, Giza, Dweik, M., Stringer, R. C., Dastider, S. G., Wu, Y., Almasri, M. and Barizuddin, S. (2012); Specific and targeted detection of viable *Escherichia coli* O157:H7 using a sensitive and reusable impedance biosensor with dose and time response studies. Department of Dairy Science, National Research Center, Talanta 94(0): 84-89.

Elias, K., Hussein, D., Asseged, B., Wondwossen, T., and Gebeyehu, M. (2008); Status of bovine tuberculosis in Addis Ababa dairy farms. *Rev. Sci. Tech. Off. int. Epiz.*, 27:915-923

Elmahmood, A.M./ Doughari, J.H.(2007). 'Microbial quality assessement of Kuhum-Zaki beverage sold in Girei town of Admawa State, Nigeria'. *African Journal of Food Science;* 011–015.

EL-zubeir IEM, Ahmed, M I.(2007). 'The hygienic quality of raw milk produced by some dairy farms in Khartoum-Sudan'. *Journal of Microbiology*, 2: 988-991.

ENA (2004). Milk, Dairy products loss of five African, Middle East countries stands At 90 Mln. USD, Ethiopian News Agency (ENA), Addis Ababa, 10/22/2004.

Enb, A, Abou Donia, M.A, Abd-Rabou, N.S, Abou Arab, A.A.K, E Senaity, M H. (2009). Chemical composition of raw milk and heavy metals behavior during processing of milk products. In *Global Veterinaria*, 3(3):268-275.

Esron, D., Karimuebo E, Lughano T, Kusiluka RH, Melegela Aglowisye M, Kapaa M, Kalvin S. (2005). 'Study on Mastitis, milk quality and health risk associated with consumption of milk from pastoral herds in Dodoma and Morgora region'. *Tanzania, Journal of Veterinary Science* 6: 213-221.

ES ISO (6785:2001). Milk and Milk products- Detection of Salmonella; Microbiology of food and animal feeding stuff-horizontal method for the detection of salmonella.ISO-6785, Geneva.

European Commission (1992). 91/180/EEC. Commission decision laying down certain methods of analysis and testing of raw milk and heat treated milk. *Official Journal of European Communities;L93:1-48.*

Fadda, M.E., Mossa, V., Pisano, M.B., Deplano, M., Cosentino, S.(2004). 'Occurrence and characterization of yeasts isolated from artisanal Fiore Sardo cheese'. *International Journal of Food Microbiology*, 95; 51–59.

FAO (2011). Global food losses and food waste: Extent causes and prevention. Study conducted for the International Congress, SAVE FOOD, at Interpack 2011, Düsseldorf, Germany.

FAO(2008). Milk hygiene in milking, milk production hygiene and udder health. FAO Animal Production and Health Papers-78. FAO Corporate Document Repository.(CDR), pp.1-7.

Fatine, H., Abdelmoula, E, Doha B, Hinde, H. (2012). 'Bacterial Quality of Informally Marketed Raw Milk in Kenitra City, Morocco'. *Pakistan Journal of Nutrition*;11(8): 662-669.

FDA (2012). Bad bug book: Food-borne pathogenic microorganisms and natural toxins handbook, 2nd ed.US Food and Drug Administration, Silver Spring, p.87–92.

Felleke, G. (2003). Milk and Dairy Products, Post-harvest Losses and Food Safety in Sub-Saharan Africa and the Near East. A Review of the Small Scale Dairy Sector-Ethiopia. FAO Prevention of Food Losses Program. FAO, Rome, Italy.

Feldmann, M., Zimmermann, A., Hoedemaker, M.(2006). Influence of milking techniques, milking hygiene and environmental hygiene parameters on the microbial contamination of milking machines. Dtsch Tierarztl Wochenschr; 113 (7): 274- 281.

Fikrineh, N., Estefanos T. and Tatek W.(2012). 'Microbial Quality and Chemical Composition of Raw Milk in the Mid-Rift Valley of Ethiopia'. *African Journal of Agricultural Research,*7,4167-4170, Available online at http://www.academicjournals.org/AJAR, DOI: 10.5897/AJAR12.830, ISSN 1991-637X ©2012 *Academic Journals.*

Francesconi, G.N.(2006). Promoting milk quality of cooperative smallholders: Evidence from Ethiopia and implications for policy. In proc. of the 14th Annual conference of the Ethiopian Society of Animal Production (ESAP), Part II: Technical papers, held on September 5-7, 2006, Addis Ababa, Ethiopia. pp 31-41.

Frank JF, Hassan AN.(2003). Microorganisms associated with milk. In: Roginski H, Fuquay JW, Fox PF. (eds.). *Encyclopedia of dairy sciences.* London, UK. Academic Press, Elsevier Science,: 1786-1796.

Garedew L, Berhanu A, Mengesha D, Tsegay G.(2012). Identification of gram-negative bacteria from critical control points of raw and pasteurized cow milk consumed at Gondar town and its suburbs, Ethiopia. BMC Public Health; 12:950.

Gebrewold A, Alemayehu M, Demeke S, Dediye S and Tadesse A.(2000). Status of Dairy Research in Ethiopia. In: The role of village dairy co-operatives in dairy development. Small holder dairy development project (SDDP), Proceeding of Ministry of Agriculture (MOA), Addis Ababa, Ethiopia.

German Technical Cooperation (GTZ).(2006). Value Chain Analysis and "Making Markets Work for the Poor" (M4P) –Poverty Reduction through Value Chain Promotion. Eschborn: GTZ.

Gete, Z., Trutmann, P, and Aster, D.(2007). Fostering new development pathways: Harnessing rural-urban linkages (RUL) to reduce poverty and improve environment in the highlands of Ethiopia. Proceedings of a planning workshop on thematic research area of the global mountain program (GMP) held in Addis Ababa, Ethiopia, August 29-30, 2006. Global Mountain Program. *pp 235.*

Ghada, ZA. (2005). 'Mineral composition of raw cow milk collected from different dairy farms in Egypt'. *African Journal of Food Science and technology*; 4(3):201-209.

Haasnoot, W., N.G.E. Smits, A.E.M. Kemmers-Voncken and M.G.E. Bremer,(2004). 'Fast biosensor immunoassays in milk of ewes and goats'. *Journal of Dairy Science,* 71: 322-329.

Hahn, G. (1996). Pathogenic bacteria in raw milk situation and significance. Proceeding of Bacteriological quality of raw milk. International Dairy Federation, Brussels (Belgium), pp: 67-83.

Hassan, I.P. (2005). Quality Assurance of Various Dairy Products. MSc Thesis, Department of Chemistry, University of Peshawar, Pakistan: 86-89.

Haug, A., Hostmark, A.T. and Harstad, O.M.(2007). Bovine milk in human nutrition- a review. Lipids in Health and Disease 6:25.

Hempen, M., Unger, F., Münstermann, S.,Seck, M.T., Niamy, V.(2004). The hygienic status of raw and sour milk from smallholder dairy farms and local markets and potential risk for public health in The Gambia, Senegal and Guinea. Animal Health Research Working Paper 3. ITC (International Trypanotolerance Centre), Banjul, The Gambia, 54 pp.

International Dairy Federation (IDF) (2008). Minerals in milk and milk products 1/8,2/8

IFCN. (2006). Dairy Report (2006). For a better understanding of milk production world-wide. International Farm Comparison Network (IFCN) Dairy Research Center, Kiel, Germany.

Imami meybodi, M.A., Mahdavi adali, H.R., Shokohmand, M., Karimi, A. and Mophidi, M.R. (2010). The survey of microbial load and raw milk additives in Yazd province. *The fourth Congress on animal science. Iran.*

Isabel, S., Josph VR, Carla S, Jose MS, Jord M. (2009). Microbial contamination milk and dairy products from resturants Spain. Foodborn Pathogens and Disease, 6:10.

ISO (1998). Microbiology of food and animal feeding stuff-horizontal method for the detection of salmonella.ISO-6579, Geneva.

ISO (1999). Microbiology of food and animal feeding stuff-horizontal methods for staphylococci.ISO 6888-1, Geneva.

Javaid, SB, Gadahi JA, Khaskeli M, Bhutto M B, Kumbher S and Panhwar A H.(2009). 'Physical and chemical quality of market milk sold at Tandojam, Pakistan'. *Pakistan Vet. J. 29*(1): 27-31.

Jayarao, BM, Pillai SR, Sawant AA, *et al.* (2004). 'Guidelines for monitoring bulk tank milk somatic cell and bacterial counts'. *Journal of Dairy Science*; 87:3561–3573.

Jayarao, B. M., Donaldson, S. C., Straley, B. A., Sawant, A. A., Hegde, N. V. and Brown, J. L. (2006). 'A survey of food-borne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania'. *Journal of Dairy Science* 89: 2451-2458.

Jones, W.P., Waston, RP, Wallis ST.(2004). Salmonellosis. In Andrews HA, Blowey, W.R., Boyd H, Eddy GR, Bovine Medicine: Diseases and Husbandry of Cattle. 2nd ed. UK: Blackwell Science. pp. 215-230.

Kagkli, DMM, Vancanneyt P, Vandamme CH, Cogan TM. (2006). 'Contamination of milk by enterococci and coliforms from bovine faeces'. *Journal of Applied Microbiology,* 1364-507.

Kaloreu, D., Shanmugam, Y., Kurkure, N. Chousalkar, K. and Barbuddhe, S.(2007). 'PCR based detection of genes encoding virulence determinants in *Staphylococcus aureus* from bovine sub clinical mastitis cases'. *Journal of Veterinary Science*, 8: 151-154.

Kaper, J.B., Nataro J.P. and Mobley H.L.T.(2004). Pathogenic *Escherichia coli*. Nat. Rev. Microbiol., 2: 123-140.

Karmen GT, Slavica G T.(2008). The microbiological quality of raw milk after introducing the two day's milk collecting system. *Acta Agr Slovenica* 92 (1):61-74.

Kedija, H., Azage, T., Mohammed, Y. K., and Berhanu, G., (2008). Traditional cow and camel milk production and marketing in agropastoral and mixed crop–livestock systems: The case of Mieso District, Oromia Regional State, Ethiopia. IPMS (Improving Productivity and Market Success) of Ethiopian Farmers Project Working Paper 13. ILRI (International Livestock Research Institute), Nairobi, Kenya. 56:1-3

Keefe, G, Elmoslemany A.(2007). Consumer acceptance of fluid milk after raw milk selection using bulk tank bacteriologic and somatic cell count criteria. Pp 218–219 in Natl. Mastitis Counc. Annu. Mtg. Proc., San Antonio, TX. Natl. Mastitis Counc. Inc., Madison, WI.

Khan, M. T. G., Zinnah, M. A., Siddique M. P., Rashid M. H. A., Islam and, M. A. Choudhury and K. A.(2008). 'Physical and Microbial Qualities of Raw Milk Collected from Bangladesh Agricultural University Dairy Farm and the Surrounding Villages, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh', *Bangladesh Journal Veterinary Medicine* vol., 6, 217–221.

Leitner, G., Silanikove, N., Jacobi, S., Weisblit, L., Bernstein, S., and Merin, U.(2008). 'The influence of storage on the farm and in dairy silos on milk quality for cheese production'. *International Dairy Journal; 18(2):109-113.*

Li-Qiang, Q., Xiao-ping, W., Li, W., Tong, X., and Tong, W.(2009). 'The minerals and heavy metals in cow' s milk from china and Japan'. *Journal of health science,* 55:300-305.

Loir Y, Baron F, Gautier M.(2003). *Staphylococcus aureus* and food poisoning. Genetics and Molecular Research 2(1):63–76

Lues, JFR, De Beer H, Jacoby A, Jansen KE, Shale K.(2010). 'Microbial quality of milk, produced by small scale farmers in a peri-urban area in South Africa.' *African J Microbiology Res*;4(17):1823- 1830.

Lundy, M., Gottret, M., Cifuentes, W., Ostertag, C., Best, R., Peters, D., Ferris, S.(2004). Increasing the competitiveness of market chains with smallholder producers. Field Manual 3. The Territorial Approach to Rural Agro-enterprise Development. Centro Internacionale de Agricultura Tropical, Cali, Colombia.

Mekonnen, A.(2009). Isolation and Identification of *Staphylococcus* Species from Cottage Cheese *(Ayib)* and Raw Bovine Milk in Debre Zeit, Ethiopia, MSc. Thesis Addis Ababa University, Faculty of Veterinary Medicine Debre Zeit, Ethiopia.

Mekuria, A., Asrat D., Woldeamanuel Y. and Tefera G.(2013). 'Identification and antimicrobial susceptibility of *Staphylococcus aureus* isolated from milk samples of dairy cows and nasal swabs of farm workers in selected dairy farms around Addis Ababa, Ethiopia.' *African Journal of Microbiology Research;*7(27):3501-3510.

Mizumoto, N., K. Sasai, H. Tani and Baba E.(2005). Specific adhesion and Invasion of Salmonella enteritidis in the vaginas of laying hens. Veterinary Microbiology ,111(1-2):99-105. DOI:10.1016/j.vetmic2005.09.006.PMID:16242866.

Mohammed, A M A, Simeon E, Yemeserach A.(2004). Dairy Development in Ethiopia. EPTD Discussion Paper No.123.International Food Policy Research Institute, NW Washington, D.C

Monsen, E. R., Hallberg, L., Layrisse, M., Hegsted, D. M., Cook, J., Mertz, W. & Finch, C. A. (1978). 'Estimation of available dietary iron'. *The American Journal of Clinical Nutrition*, 31, 134-141.

Montville, T. J, Matthews K R.(2008). Food microbiology: An introduction. 2nd ed, ASM Press, Washington D.C.

Mosu, S., Megersa M., Muhie Y., Gebremedin D. and Keskes S. (2013). 'Bacteriological quality of bovine raw milk at selected dairy farms in Debre Zeit town, Ethiopia'. *Comprehensive Journal of Food Sciences and Technology Research* ;1(1):1-8.

Murphy, SC, Boor K J.(2003). Basic dairy Bacteriology. Microbiological quality defects in fluid milk products: The evaluation of shelf life. Cornell University, Ithaca, NY.

Nandy, SK., Bapat P, Venkatesh KV.(2007). Sporulating Bacteria Prefers Predation to Cannibalism in Mixed Cultures. FEBS Lett. 581: 151-156.

Nanu E, Latha C, Sunil B, et al. (2007). ' Quality Assurance and public health safety of raw milk at the production point'. *American Journal of Food Technology*; 2:145-52.

Nebiyu RG.(2008).Traditional and improved milk and milk products handling practices and compositional and microbial quality of raw milk and buttermilk in Delebo water shade of Wolayita zone. MSc. Thesis, Hawassa University, Ethiopia.

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

Olatunji, EA., Jubril AE, Okpu EO, Olafadehan OA, Ijah UJ and Njidda AA.(2012). Bacterial Assessment and Quality Analysis of Raw Milk Sold in Gwagwalada Area Council of the Federal Capital Territory (FCT) Abuja, Nigeria. Food Science and Quality Manage. 7,ISSN 2224-6088 (Paper) ISSN 2225-0557.

Olfa, S B., Rouaa L, Imène F, Hamadi A and Mohamed A A. (2013). 'Detection of *Escherichia coli* in unpasteurized raw Milk', *International Journal of Agricultural and Food Science*, 3(2): 53-55

Oliver, SP., Jayarao BM, Almeida R A. (2005). Food borne pathogens in milk and the dairy farm environment. Food safety and public health implication. Food borne Pathog. Dis., (2): 115-129.

Ombui, J.N., Kaburia, H.F., Macharia, J.K. and Nduhiu, G.(1994). 'Coliform counts and *Escherichia coli* in raw commercial milk from dairy farmers in Kiambu District, Kenya'. *East African Medical Journal,* 71: 635-639.

Padulosi, S., Noun J, Giuliani A, Shuman F, Rojas W, Ravi B. (2004). Realizing the benefits in neglected and underutilized plant species through technology transfers and human resources development. In: Proceedings of the Norway/UN Conference on Technology Transfer and Capacity Building, 23-27 May, 2004. Trondheim, Norway. Pp. 117-127.

Park, Y W.(2006). Goat milk-chemistry and nutrition. In: Park, Y W , Haenlein, G F W (eds.), Handbook of Milk and Non-bovine Mammals. Oxford: Blackwell Publishing Professional, UK, 34-58.

Perko, B.(2011). Effect of prolonged storage on microbiological quality of raw milk, Mljekarstvo ;61 (2): 114-124.

Popescu, A. and Angel, E.(2009). Analysis of milk quality and its importance for milk processors. *Lucrări Ştiinţifice Zootehnie Şi Biotehnologii, Timisoara,* 42(1):501-503.

Quinn, PJ., Carter ME, Markey B, Carter GR (1999).Clinical Veterinary Microbiology. Moss by International Limited, Spain, 118-143:209-242

Radostits, OM., Gay CC, Hinchcliff KW, Vonstable PO. (2007). Veterinary Medicine, a Text Book of the Disease of Cattle, Horses, Sheep, Pigs and Goats.10th ed. London: Saunders Elsevier. pp. 896 - 921.

Ramesh, C., Chandan(2006). Manufacturing Yogurt and Fermented Milks, 6th ed., Blackwell Publishing Ltd, Oxford, UK, pp.7-40.

Randolph, H(2006). Identifying spoilage bacteria and potential shelf- life problems. *Dairy Food*/Find Article 1-3.

Rehrahie, M., Yohannes, G. (2000). Effect of supplementary concentrate feeds with different ingredients for local and crossbred cows on major milk constituents. Proceedings of 7th annual conference of Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, May 26-27 1999. pp. 263-267.

Rezaei, MF., Karimi, M Yahyaei, H Javdani, A Shahabi and Farahi, A (2013). The survey of microbial total count and prevalence of *Escherichia coli* in raw milk in Markazi Province, Iran. Res. Opin. Anim. Vet. Sci., 3(12), 474-477.

Richardson, GH. (1985). Standard Methods for the Examination of Dairy Products. 15th ed. American Public Health Association. Washington, D. C, pp. 168-196.

Ruegg P L.(2003). 'Practical food safety interventions of dairy production.' *Journal of dairy Science*; 86(12): 1-9.

Salandra, G., Goffredo, E., Pedarra, C., Nardella, M., Parisi, A., Dambrosio, A., Quaglia, N., Celano, G and Normanno, G.(2008) 'Occurrence, characterization and antimicrobial resistance pattern of *Staphylococcus* species isolated from dairy products in southern Italy'. *International Journal of Food Microbiology*, 9: 327-360.

Saxena, M.M., Katpatal ,B.G. and Pandey, H.S.(1997). 'Study of milk constituent and their yield in Holstein- Friesian cows'. *Indian Journal of Animal production and Management* 13(3):127-130

Shekhar C,Motina E, Kumar S.(2010). 'Microbiological Quality of Raw Milk and its Public Health Significance'. *Journal of Dairying, Foods and Home Sciences* 29:19-25.

Shojaei ZA and Yadollahi A. (2008). 'Physicochemical and microbiological quality of raw, pasteurized and UHT milks in shops'. *Asian Journal of Scientific Research*, 1:532-538.

Singh, P. and Prakash A.(2008). Isolation of *Escherichia Coli, Staphylococcus aureus* and *Listeria monocytogenes* from milk products sold under market conditions at Agra region *Acta agriculturae Slovenica*, 92(1); 83-88.

Sintayehu, Y., Beyene .F, Tegegne A, Gebremedhin B(2008). Dairy production, processing and marketing systems of Shashemene- Dilla area, South Ethiopia. Improving

Productivity and Market Success (IPMS), International Livestock Research Institute (ILRI),Addis Ababa, Ethiopia.

Smiddy, MA., Martin JE, Huppertz T, Kelly A L. (2007). 'Microbial shelf-life of high-pressure homogenized milk'. *International Dairy Journal* 17: 29-32.

Smith, K. (2007). Food borne pathogenic microorganisms and natural toxins. *Food and Drug Administration, Center for Food Safety and Applied Nutrition*, 10: 119-150.

SNV. (2008). Netherlands Development Organization Study on Dairy Investment Opportunities in Ethiopia.

Soares, V.A., Kus, M.M., Peixoto, A.C., Carrocci, J.S., Salazar, R.F., Filho, H.I., (2009). Determination of nutritional and toxic elements in pasteurized bovine milk from Vale do Paraiba region(Brazil). *Food Control,* 21: 45-49.

SPSS (Statistical Procedures for Social Sciences) (2007). SPSS (Version 16). Statistical. SPSS BI Survey Tips.Inc. Chicago, USA.

Tassew, A., and Seifu, E. (2011). 'Microbial quality of raw cow's milk collected from farmers and dairy cooperatives in Bahir Dar Zuria and Mecha district, Ethiopia'. *Agriculture, Biology J. N. Am.* 2(1): 29-33.

Tebug, S. F., Kasulo, V., Chikagwa-Malunga S., Wiedemann S., Roberts D. J. and Chagunda M. G.(2012). Smallholder dairy production in Northern Malawi: production practices and constraints. *Tropical Animal Health and Production*, 44 (1), 55–62.

Tesafye, L., Puskur R., Azage T. and Hoekstra D.(2008)."Exploring innovation capacity in the Ethiopian dairy systems." Paper presented on the international conference on: Advancing Agriculture in Developing Countries through Knowledge and Innovation, International Food Policy Research Institute, Addis Ababa.

Tesfaw, L., Taye B., Alemu, S., Alemayehu, H., Sisay, and Negussie, H. (2013). Prevalence and antimicrobial resistance profile of Salmonella isolates from dairy products in Addis Ababa, Ethiopia'. *African Journal of Microbiology Research*;7(43):5046-5050.

Tesfay, T., Kebede, A., and Seifu, E. (2013).'Quality and Safety of Cow Milk Produced and Marketed in Dire Dawa Town, Eastern Ethiopia'. *International Journal of Integrative Sciences, Innovation And Technology Section B*;(2)6: 01-05.

Teshager, A., Belay, D., and Taye, T. (2013). Smallholder Cattle Milk Production, Utilization and ,Marketing Pattern in Different Agro-Ecological Districts of Ilu Aba Bora Zone, Southwestern Ethiopia'. *American-Eurasian Journal of Scientific Research;* 8(1): 24-30, 2013 ISSN 1818-6785 ;DOI: 10.5829/idosi.aejsr.2013.8.1.6671.

Tola, A., Ofodile, LN., and Fikadu, B. (2007). Microbial quality and chemical composition of raw whole milk from Horro cattle in East Wollega, Ethiopia. Available on: www.ajol.info/index.php/ejesc/article/View/41995

Tola, A. (2002). Traditional milk and milk products handling practices and raw milk quality in eastern Wollega. MSc Thesis. Alemaya University, Ethiopia.

Ulfina, G., Jiregna, D., Alganesh, T., Shiv, P and Late Mulugeta, K. (2013). 'Dairy Production Potential and Challenges in Western Oromia Milk Value Chain, Oromia, Ethiopia'. *Journal of Agriculture and Sustainability* ISSN 2201-4357 (2)1: 1-21

Urban Dairy Sector Study, (2006).Addis Ababa City Administration, Trade and Industry PP.2-3.

Vahedi, M. Nasrolahei, M., Sharif, M., Mirabi, A.M. (2013). 'Bacteriological study of raw and unexpired pasteurized cow's milk collected at the dairy farms and super markets in Sari city in 2011.' *Journal of preventive medicine and hygiene*; 54: 120-123.

Van Kessel, J. S., Karns, J. S., Gorski, L., McCluskey, B. J., Perdue, M L. (2004). 'Prevalence of salmonella, *Listeria monocytogenes*, and fecal coliforms in bulk tank milk on U.S. dairies'. *Journal of Dairy Science* 87, 2822-2830.

Vasanthakumari, R. (2009). Practical Microbiology. BI Publications Prt Ltd, New Delhi; 57.

Vermeulen, S., Woodhill J., Proctor, FJ., Delnoye, R. (2008). Chain-wide learning for inclusive agro food market development: A guide to multi stakeholder processes for linking small scale producers with modern markets. International Institute for

Environment and Development, London, UK, and Wageningen University and Research Centre, Wageningen,The Netherlands.

Weigel, R., B. Teferedegne, D., Suh, Barber, and D., Isaaoson, R. and White, B. (2004). Comparison of pulsed field gel electrophoresis and repetitive sequence polymerase chain reaction as genotyping methods for detection of diversity and inferring transmission of salmonella. *Veterinary Microbiology*;100(3-4):205-217.

White, DG., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen S, Mcdermott, S., Waner, DD, Meng, J. (2001). 'The isolation of antibiotic-resistant Salmonella from retail ground meats.' *New England Journal of Medicine* 345(16):1147-1154.

Worku, T., Negera, E., Nurfeta, A. and Welearegay, H.(2012). 'Microbiological quality and safety of raw milk collected from Borana pastoral community, Oromia Regional State'. *African Journal of Food Science and Technology;*3(9): 213-222.

Wouters, J.T.M., Ayad, E.H.E., Hugenholtz, J., Smit, G.(2002). 'Microbes from raw milk for fermented dairy products'. *International Dairy Journal;* 12, 91–109.

Yagoub, SO., Awadalla, NE., El Zubeir, IEM.(2005). 'Incidence of some potential pathogens in raw milk in Khartoum North (Sudan) and their susceptibility to antimicrobial agents.' *Journal of Animal Veterinary Advance*; 4:341-344.

Yan, S., Pendrak, M., Abela-Ridder, B., Punderson, J., Fedorko, D. and Foley, F. (2003). An overview of Salmonella typing public health perspectives. Clin. App. Imm. Rev., 4(3):189-204. DOI: 10.1016/j.cair.2003.11.002.

Yip, R. (1994). Iron deficiency: contemporary scientific issues and international programmatic approaches. *The Journal of Nutrition;* 124, 1479.

Yitaye, AA., Maria, W., Azage, T. and Zollitsch, W. (2009). Performance and limitation of two dairy production System in the North western Ethiopian highlands. Tropical Animal production and Health;41(7):1143-1150.

Zadoks, RN., Gillespie, BE., Barkema, HW., Sampimon, OC., Oliver, P., Schukken, Y. (2007). 'Comparison of the Etiology of Environmental Mastitis in two herds of Dairy cows'. *Slovak Journal of Animal Science;*40(3): 132-140.

Zelalem, Y. (2003). Sanitary conditions and microbial qualities of dairy products in urban and peri-urban dairy shed of the central Ethiopia. DEA. Lyon, France.

Zelalem, Y., Yohannes, G., Gizachew, B., Alemu, GW. and Sendros, D. (2004). Major milk chemical composition and feed dry matter intake of multiparous Boran cows as affected by level of milk production under bucket feeding and partial suckling calf rearing methods. Proceedings of the 11th annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, August 28- 30, 2003, pp. 397-402.

Zelalem, Y., Faye, B., and Loiseau, G .(2005). 'Microbial properties of butter and Ethiopian cottage cheese (Ayib)'. *Ethiopian Veterinary Journal;*9(1):43-57.

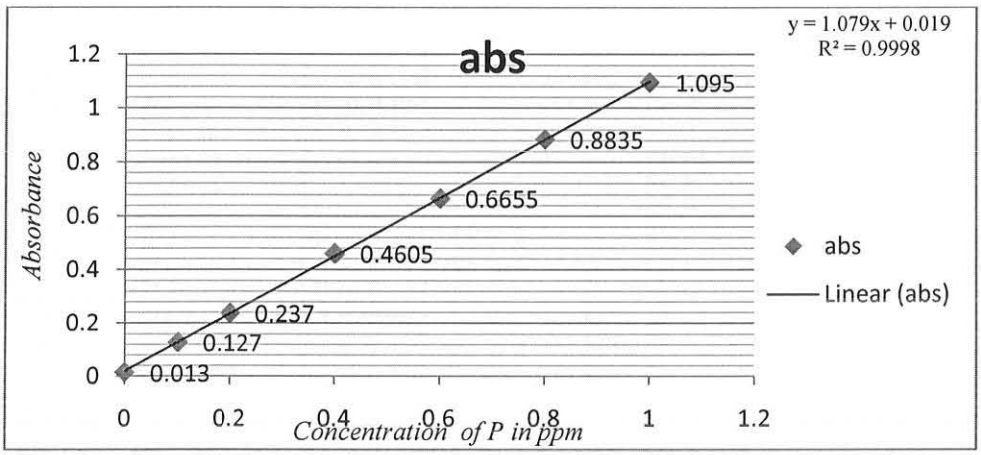
Zelalem, Y. and Faye, B. (2006). 'Handling and Microbial Load of Cow's Milk and Irgo - Fermented Milk Collected from Different Shops and Producers in Central Highlands of Ethiopia'. *Ethiopian Journal of Animal Production;* 6(2):67-82.

Zelalem, Y., Faye, B. and Loiseau, G. (2007). Occurrence and distribution of species of Enterobacteriaceae in selected Ethiopian traditional dairy products: A contribution to epidemiology. Food Control 18 (2007) 1397–1404.

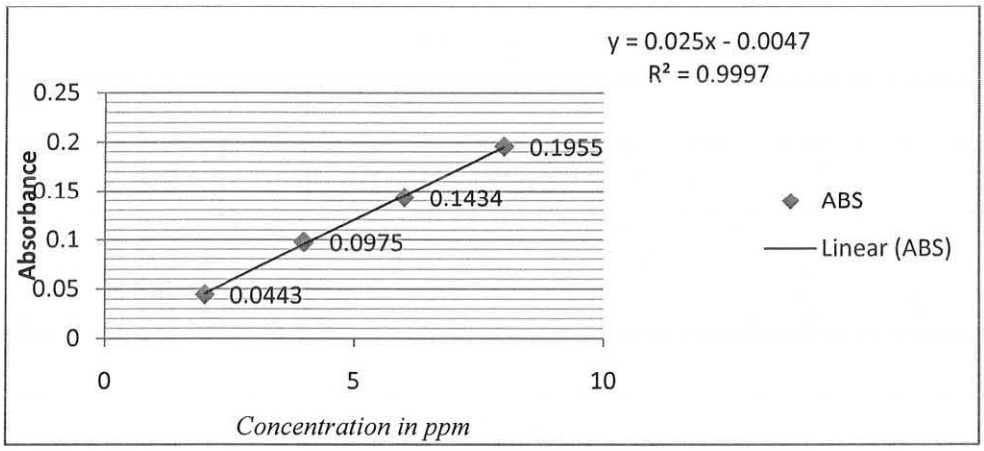
Zucali, M., Bava, L., Tamburini A., Brasca, M., Vanoni L. and Sandrucci, A. (2011). 'Effects of season, milking routine and cow cleanliness on the bacterial and somatic cell counts of bulk tank milk.' *Journal of Research;* (4): 436-441.

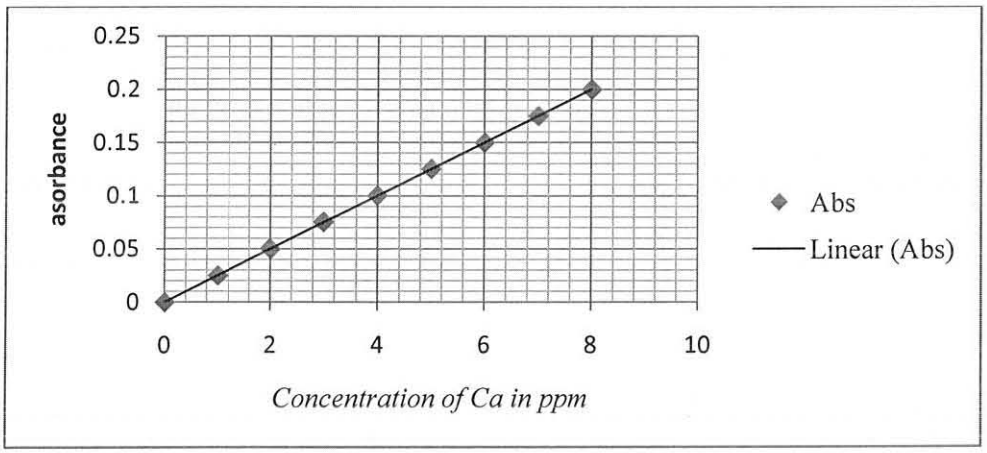
Appendix 1

Standard calibration curve for phosphorus



Standard calibration curve for iron





Appendix 2

Questionnaire

The purpose of this questionnaire is to check safety and quality of cow milk and constraints that hinder production, milk collection, transportation and marketing of milk

Dear;

This questionnaire is prepared for the purpose of assessing milk quality, constraints that hinder production, level of knowledge on milk production, milk collection, transportation and marketing. Therefore, I would like to acknowledge you for your good cooperation by providing real and timely information

A. General description

 # Farmer level Questions

Zone_____ Woreda _____ Kebele _____ AEZs_____

Respondent Name _____ Age_____ Sex____ Mobile No. _____

B. Barn hygiene

Are you practice barn cleaning daily? 1. yes 2. No ; bedding condition 1. yes 2. No

C. Dairy Animals performance

1. How many dairy cows are in the herd fill in the following table

No.	*Breed*	*Number of cows currently owned(2013/14) a=2, b=3, c=1, d= >3*	*2010/11 G.C a=2, b=3, c=1, d= >3*
1	Local		
2	Cross breed		
3	Exotic		

i. From how many cows on the farm is the milk pooled from presently?

1. two 2. one 3.three 4.> three

ii. How many times do you milk your cows per day?

1. Morning only 2. Morning and evening 3. Morning, mid day and evening

2. *How many months of lactation do you normally have?*

Months of lactation				
No.	*Breed*	*1=3-6 months*	*2=7-9 months*	*3=9-12 months*
1	Local			
2	Cross breed			
3	Exotic/pure breeds			

3. Do you intend to increase your level of milk production? 1. Yes 2. No

4. If yes, describe

1. It maintains food production for the household 2. It is profitable (income generation)

5. If no, indicate: 1. It is not as crop production 2. It is not profitable

D. Pre- milking udder preparation and hygiene

1. Are flanks, udder or teats washed before milking? 1. Yes 2. No

i. If yes, cleaning material used

1.Cold tap water 2. Warm River 3. Warm tap water

4. Cold river water 5. Cold well water 6. if any specify_____

2. According to question #1 if any of your choose of water type, which agent do you use?

1. Detergent 2. Disinfectant

3. soap/Omo 4. Other (specify) _____

3. Do you use teat dips? 1. Yes 2. No

i If yes, when? 1. Pre-milking 2. Post-milking 3. Both before & after milking

ii. with: 1. Iodine 2. alcohol 3. If other Specify_____

4. Do you use towel for cleaning and drying teat and udder? 1. Yes 2. No

5. How often towel washed after cleaning teat/udder? 1. yes 2.No , If yes when?

1. After each usage using cold water 2. After each usage using warm water

6. Do you wash hands before milking? 1. Yes 2. No

7. Have you ever wash your hands between milking cows? 1. Yes 2. No

E. Milking technique, milk cooling and transport ?

1. Milking procedure used: 1. Hand 2. Machine 3. Both

2. Milking frequency per day: 1. Once 2. Twice 3. >3

3.Do you use traditional flavor agent to clean milk transporting equipment? 1. yes 2. No

If yes, List _____, _____,_____&_____

4. Equipment used for milking: 1. plastic 2. steelness stain 3. metal can

4. clay pot 5. specify other_____

5. Equipment used for storage before transportation: 1. plastic 2. steelness stain 3. metal can

4. clay pot 5. Aluminum can 6. specify other_____

6. Equipment used for transportation: 1. plastic 2. steelness stain 3. metal can

4. clay pot 5. specify other_____

7. What type of plants do you use for smoking milking and milk storing equipments, list at their rank

1. _____2. _____ 3. _____4. _____

8. Do you use cooling system for milk? 1. Yes 2. No If yes, how?

1. refrigerator 2. At room temperature 3. Traditional cooling system 4.others _____

F. Main challenges of dairy production, milk transportation and processing?

1. What are the main constraint out of the followings for your dairy production?

1. Feed shortage 2. High feed prices 3. Disease

4. High medication cost 5. Shortage of land for grazing or forage development

6. Lack of capital 7 Inefficient breeding services 8. Market availability 9. All

2. Could you rank the most important constraints which dairy farming/milk production?

1. Feed shortage ___ 2. Diseases ___ 3. Shortage of land ____ 4 Capital ___ 5. Market ___

i. If animal disease, list major ones in the area

1. Anthrax 2.Mastitis 3.brucellosis 4. Contagious bovine pleura pneumonia(CBPP)

ii. Transportation problem, if mention : 1. distance 2. availability 3. being costly

iii. Market demand/supply:

1. Price fluctuation due to fasting season 2. Milk quality/adulteration

3. Distance from production area 4. Specify Others_____

G. Use of technology for dairy production?

1. Have you get a government support that helps to expand your dairy farm ? 1. Yes 2. No
2. Have you get training on hygienic production, transportation & marketing system of milk?

1. Yes 2. No

3. If yes,

i. Who was the responsible for the training?

1. Government 2. NGO's 3. cooperatives 4. Private company

5. Processing plants 6. Specify Others_____

ii. How many times did you have had the training? 1. twice 2. Only one times

3. four times 4. > 4 times

iii. Does training brought any improvement in milk production and marketing system at your household level? 1. yes 2. No

4. If not get training, what were the reasons_____

H. Milk production, Utilization, marketing and processing

❖ ***i .Milk production and utilization***

1. Which gender group plays a great role in dairy production?

1. Males 2. Females 3. Both almost equally

2. How much milk is produced per cow per day in your herd on the average?

1. 1-5 liters 2. 6-10 liters 3. >10 liters 4. 15-20 liters 5. >20 liters

3. At what season of the year do you get more milk?

1. dry season 2. wet season 3. short rain season

4. At what season of the year do you get the lowest milk yield?

1. Dry season 2. Wet season 3. Short rainy season

5. How is the milk consumed?

1. Alone 2. With meals 3. As an additional food 4. Others specify_____

6.Pattern how is it utilized?

No.	*Milk Utilization pattern*	*Amount in liters/day*	*% from the total produced*
1.	Total Milk produced		
2.	For calf feeding		
3.	For home consumption		
4.	For processing		
5.	For sales		
6.	For other purposes		

7. Are there seasonal variations in consumption pattern? 1. Yes 2. No

8. If yes, indicate _____

9. Do you process your milk? 1. Yes 2. No

10. If yes, at what time interval do you process the milk?

1. Every day 2. Every two intervals 3. Every week 4. Specify if other_____

11. What materials do you use to process the milk? 1. Clay pot 2. Gourd 3. plastic 4. Other_____

12. Do you use any new method to improve the quality of milk during consumption/marketing?

1. yes 2. No

3. If yes, specify it_____

❖ ***ii. Milk marketing***

1. Do you practice milk marketing? 1. yes 2. No

2. If yes, for whom do you sell your milk?

1. To local market 2. To milk collection center 3. private processing plant

4. retail shops 5. hotels and restaurants 6. If others_____

3. If your answer on question "Q #2" is "to processing plant" how often?

1. Every day 2. Every other day 3. Other (specify) _____

4. In what form do you market/sale your milk ?

1. Whole Milk 2. Fermented Milk 3. Butter Milk 4. If other_____

5. What criterion do you mostly use in selecting your milk marketing out let?

1. Price 2. Distance 3. Reliability 4. Long term contract 5. Milk thickness

6. How do you transport milk to market?

1. By vehicle 2. By cart horses or donkeys 3. By loading directly on horse or donkey back

4. By bicycle 5. On foot 6. Specify if other_____

7. How long do you travel to reach market/milk collection centers?

1. By vehicle_____ min/hr 2. On foot by holding milk_____ min/hr

3. By pack animals_____ min/hr 4. By cart-horse/donkey_____ min/hr

8. At what season of the year do you sell more amount of milk? 1. dry season

2. wet season (long rainy season) 3. short rain season

9. During which holidays do you sale/market more milk and milk products with better price?

List in order: 1. charismas 2.Easter 3.New year 4. Meskel 5. specify if other

10. What mechanism do you use for determining the quality of milk in the market/collection center? By

1. testing 2. Smelling 3. Color/appearance of the product 4. specify if other_____

11. Is that milk production & marketing have any significant effect on your household food security?

1.Yes 2. No

If yes, 1. for purchasing food crop 2. student's school fee 3. saving 4. purchasing land

12. Is there any period you have problem of marketing your milk?

1. Yes 2. No

13. If yes, which month ? 1. Fasting months 2. In any month in the year 3. In wet season 4. other

14 . what are the major challenges of milk marketing?

1. price fluctuation 2. transportation problem 3. lack of constant customer flow

4. Lack of technological equipment for transportation 5. If other specify_____

❖ ***iii. Household's Cooperative Membership and milk transportation to collection center***

1. Are you the member of any dairy/milk cooperatives in the area? 1. Yes 2. No

If yes, what are their roles in milk marketing and timely collection?

_____

If No, what is the reason?_____

2. How often do you bring milk to the collection center?

1. Twice a day 2. Once a Day 3. at one day interval 4. Other_____

3. How do you store the milk until it is transported to the collection center?

1. in refrigerator 2. Open 3. Closed 4. in water bath 5. Other_____

4. How long did it take for the milk to get to the collection center since the cow(s) was(were milked)? 1. < 6 hrs 2. B/n 6 & 12 hrs 3. > 12 hrs

5. How many liters of milk are brought into the collection center each time on average?

1) 3 *lt* 2) 4 *lt* 3) 5 *lt* 4) 2 *lt* *5)>5lt*

❖ ***iv. Dairy cow diseases***

1. How often do milking cows checked for diseases?

1. Once a month 2. Every quarter 3. Random 4. Is not common practice

2. Are any records kept? 1. Yes 2. No If yes, Specify it_____

3. Do you have incidence of human beings infected with any of the diseases? 1. Yes 2. No

4. If yes, which disease 1. Anthrax 2. mastitis 3. Brucellosis

5. Do you use any traditional or herbal remedies for your dairy cows? 1. Yes 2. No

6. If yes why? 1. Vet. Services are not available 2. Vet costs are high 3. If other specify_____