ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE
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Assessment of Dairy Food Environment, Physicochemical Properties, and Microbial Safety of Milk and Cottage Cheese across the Value Chain in Oromia Region, Ethiopia

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A Thesis Submitted to the College of Natural and Computational Science of Addis Ababa University in Partial fulfillment of the requirement for the degree of Master of Science in Food Science and Nutrition

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"Assessment of Dairy Food Environment, Physicochemical Properties, and Microbial Safety of Milk and Cottage Cheese across the Value Chain in Oromia Region, Ethiopia"

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STATEMENT OF THE AUTHOR

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Abstract

Milk and milk products are nutrient rich foods, supplying energy and high-quality protein with a range of essential micronutrients. But, it becomes a health risk to the consumers if not handled properly due to high perishability and vulnerability to microorganisms. The present study was conducted in a cross-sectional study design to evaluate the dairy food environment and assess physicochemical properties, microbial safety of milk, and cottage cheese across the value chain in the Oromia region. A total of 65 milk and cottage cheese were collected for laboratory analysis including clot-on boiling and alcohol test, acidity, pH, total solids, fat, protein, lactose, solids non-fat, phosphorus, calcium, iron, and zinc contents. Qualitative tests of *Listeria* and *Salmonella* spp. and enumeration of *Staphylococcus aureus* and total coliforms were done following the standard methods. A total of 120 dairy products consumers of the respondents were interviewed to assess the dairy food environment.

The result showed that nearly one-third and 14% of milk samples showed positive results for alcohol clot and clot on the boiling test, respectively. Statistically, there were significant differences ($p \leq 0.05$) of the physicochemical properties across the value chain. The mean of the milk acidity was 0.32 and it was lowered by 23% compared to cottage cheese. The mean of the fat contents collected from producers was 3.98% and it was reduced by 3.7, 14.6, 15.8 and 30.2% at milk collections, unions, raw milk, and pasteurized milk retailers, respectively. The overall mean of the milk ash was 0.66, but it was higher by 25% for cottage cheese. The microbial load of the raw milk was nearly higher by 50% compared to the pasteurized milk. The highest frequency of positive samples of *Salmonella* and *Listeria* spp. was found in raw milk samples collected from the union gate. The microbial load of the cottage cheese and pasteurized milk was lower compared to raw milk samples. The result of the survey indicated that above one-third of the respondents consumed dairy products frequently and the rest of them were constrained with fasting seasons. Despite the moderate dairy product availability, the majority of the study subjects responded as the price of dairy products was imbalance with their monthly income. Hence, consolidating the milk value chain to advanced formal ways and milk processing, dairy product diversification, and applying quality control system can enhance the safety and dairy food environment.

Keywords: Cottage cheese, Dairy, Environment, Food, Milk, Safety, Value Chain

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List of Abbreviations and Acronyms

APHA	American Public Health Association
ASF	Animal Source Food
ATCC	American Type Culture Collection
CSA	Central Statistical Agency
EHEC	Enterohemorrhagic *Escherichia coli*
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GTP	Gross and Transformation Plan
HTST	High Heat Short Time
IFPRI	International Food Policy Research Institute
ISO	International Organization for Standardization
LPO	Lactoperoxidase
LTLT	Long Temperature Long Time
MF	Micro-Filtration
RV	Rappaport Vassiliadis Soya Peptone Broth
SNNP	Southern Nation and Nationalities People
SPSS	Statistical Packages for Social Science
STEC	Shiga Toxigenic producing *Escherichia coli*
UHT	Ultra Heat Treatment
UNICEF	United Nations Children Fund
VTEC	Vero-toxigenic *Escherichia coli*
WTP	World Trade program

1. Introduction

1.1 Background and Justification

In Ethiopia, milk is one of the oldest foods and many people depend on its products. It is mainly produced from indigenous livestock genetic resources owned by smallholder farmers among which cattle and camels are the major ones followed by goats and sheep. Oromia is the leading milk producing region and supplies more milk to the market compared to the other three large dairy producing regions of the country (CSA, 2008; Land O'Lakes, 2010).

Milk and dairy products are nutrient-rich foods, supplying energy and high-quality protein with a range of essential micronutrients (calcium, magnesium, potassium, zinc, and phosphorus) in an easily absorbable form (Muehlhoff *et al.,* 2013). Milk minerals are crucial for human health and development and dairy processing including cheese-making and all traits involving salt-protein interactions (Franzoi *et al.,* 2017). Milk minerals play a key role in healthy human nutrition and development throughout life especially in childhood (Muehlhoff *et al.,* 2013). Dairy products are rich in nutrients that are essential for good bone health and these include calcium, protein, vitamin D, potassium, and phosphorus (Franzoi *et al.,* 2017).

Based on the availability of crossbreed cows and proximity to the capital city, the central highlands of the country serve more milk supply to urban markets of Ethiopia (Gebrewolde, 2000; FAO, 2007). Among others, cottage cheese (*Ayib*) is the most common processed milk product produced and consumed in different parts of the country and it is the basis of traditional milk processing (Zelalem , 2010). Selale, Holetta, Sebata, and Bishoftu are the most common milk-producing areas of the country and the major suppliers for Addis Ababa milk processing plants. Selale and Wolmera areas serve as the major milk supplier to the urban market due to their proximity and presence of privatized state dairy farms that use high-grade animals (>87.5 %) exotic blood) within a 100 km radius from Addis Ababa (Gebrewold *et al.,* 2000). Vernooiji *et al*. (2010) reported that 60% of the respondents in Chancho, Holetta, and Asella had crossbreed dairy cows and used improved forage and dominantly, Chancho and Holetta greatly contributed to milk supply to Addis Ababa city. In the follow of findings, compared to other cities in the country, processed milk and milk products are more distributed in Addis Ababa

where pasteurized milk, yogurt, and cottage cheese can be easily available from their supermarkets and retailer shops. This situation drives Addis Ababa city, the highest dairy product consumer (51.85 liters) compared to the national and other towns The national average per-capita consumption of milk indicated 19 kg/year compared to other African countries (27 kg/year) and 100 kg/year to the world per capita consumption (FAO, 2003).

Consumption of animal-source food is strongly linked with nutrition improvement outcomes and increasing the consumption of milk and milk products in sufficient quantity and quality is therefore desirable to achieve significantly improved nutritional status. Hence, livestock-based interventions are deemed to be a decisive strategy to reduce malnutrition, especially in Sub-Saharan Africa where half of the world's malnourished population is residing, and where diets predominantly consist of cereal or root staple crops (Sadler & Catley, 2009; Hoddinott *et al.*, 2015; Hetherington *et al.,* 2017). Beyond the nutritional value, milk and milk products are economically important farm commodities and dairy farming is an investment option for many people. Hence, the dairy value chain plays a significant role in the dairy food system by participating different actors (Pal & Jadhav, 2013). Dairy Food environment and value chain terminologies have similar concepts. But, the dairy food environment relates with availability, access, and stability of dairy products across time, while the value chains are composed of a full range of farms and enterprises and their value-adding activities, which produce agricultural raw materials and transform them into food products that are sold to final consumers and disposed of after use (Anderson & Elisabeth, 2015).

A strong food system has a positive implication on nutrition, health and well-being, and the environment. It overlaps with agricultural systems in the area of food production, but also comprises the diverse set of institutions, technologies, and practices that govern the way food is marketed, processed, transported, accessed, and consumed. In Ethiopia, the emergence of dairy value chains increased job opportunity and dairy processing, and marketing activities which increased dairy product availability (SNV, 2008). However, in Ethiopia, market channels of milk and milk products vary based on the production system and type of value-added milk products. Dairy products are channeled to consumers through both formal and informal marketing systems and milk supply chain, milk is collected from individual dairy farmers (SNV, 2008; Haile *et al.*, 2010).

However, due to the high demand and seasonality of milk supply across the value chain, dishonest producers and traders deliberately adulterate milk and its products to increase the volume which has an impact on physicochemical properties and microbial safety of milk (El-loly *et al.*, 2013).

Ayza *et al*. (2013) have reported that although middlemen play a significant role in milk and milk product delivery, the nutritional value is being affected, due to purposeful adulteration to increase the profit. Milk collectors either chill or transport milk at ambient temperature to the processing centers. The chilled milk procured from various milk collection centers is pasteurized and packed before marketed to private retailers or milk shops. Yilma (2011) has discussed milk and milk products are produced and marketed under sub-standard hygienic conditions and open markets which increase the risk of microbial contamination, although food safety and quality are a growing concern all over the world particularly from a human health point of view. O' Connor (1994) has reported earlier that traditional milk processing and utensils used for storage are often porous by making their cleaning difficult and become reservoirs for many organisms. Dairy products are highly perishable commodities and vulnerable to spoilage, and become a health risk to consumers if not properly handled from the point of production along the value chain (Swai & Scooman, 2011). Microbial contamination of milk is the major cause of milk spoilage as well as a source of health hazards to consumers (Ngasala *et al*., 2015). The presence of a high bacterial load in raw milk reduces the keeping quality of milk and other dairy products processed from the milk (Minj & Behera, 2012).

The world still faces great challenges with foodborne diseases due to contamination of food supply. It was estimated that each year there are 1.3 million cases of active diarrhea in children under five years in the developing world and a substantial number of these cases are due to microbial contaminated foods (Haglund *et al*., 1996). Accordingly, identifying critical control points to address the quality and safety of milk and milk products across the value chain considered one of the pillars in the improvement of food safety. Hence, this study was focused on the evaluation of dairy food environment, and assessment of the physicochemical properties, microbial safety of milk and cottage cheese along the value chain in the Oromia region.

1.2 Statement of the problem

In food and nutrition security, food environment determinants are key components to identify critical control points for further interventions, despite only agriculture or food production was considered as the sole solution. Food consumption may be affected by the food environment expressed by food availability, the stability of the food supply, food access, and food utilization (FAO, 2009), as well as by physiological and health status, cultural patterns, perceptions, and societal conventions, among others (WHO, 2012). Evidence indicates that consumption of animal source food is low in Ethiopia (Ahmed *et al.*, 2018) and inadequate consumption of animal source foods is related to malnutrition problems and lack of scientific information on the dairy food environment may be related to the problem of the dairy products intake and interventions.

Consumers generally need safe and good quality milk and milk products (Ayza *et al.*, 2013), even though, the consumption of raw milk and its derivatives common in Ethiopia (Zelalem, 2003), which is unsafe from a consumer health point of view as it may lead to the transmission of foodborne diseases. Although the dairy value chain plays a crucial role in transforming the dairy sector and creates an opportunity for small scale farmers, poor handling practices across the value chain reduces the final raw milk quality expected for milk processing like pasteurization or ultra-heat treatment. Pasteurization of the milk can destroy pathogenic bacteria such as *E. coli*, *Listeria monocytogenes, Salmonella* spp. *Staphylococcus aureus,* and *Yersinia enterocolitica.* But, insufficient, fault pasteurization, raw milk quality, and poor post-pasteurized milk handling practices could allow the microorganism to be found in pasteurized milk (Pal *et al*., 2014). Several scholars (Haile, 2015; Debela *et al.*, 2015; Tesfay, 2015; Alganesh, 2016; Legesse *et al*., 2017; Haftu & Degnet, 2018) conducted studies on physicochemical properties of raw milk in Ethiopia. But, research findings along the dairy products value chain for both physicochemical properties and microbial assessment may address the control points. The nutritional completeness and quality of milk are worsening across the dairy products value chain due to bad practices by value chain actors to increase the profit rather than maintaining the nutritional quality of the dairy products. Thus, identifying the critical points could help the interventions in improving milk and milk product quality. But also, the previous research results rarely, if any, reported micronutrients (calcium, phosphorus, iron, and zinc) in milk and dairy products. Thus, this study was initiated in order to address these concerns.

1.3 Objectives

1.3.1 General Objective

The objective of the finding was to assess the dairy environment and physicochemical properties and microbial safety of milk and cottage cheese across the value chain in the Oromia Region

1.3.2 Specific Objectives

- To evaluate the physicochemical properties of raw, pasteurized milk and cottage cheese across the dairy value chain
- To assess the microbial safety of raw, pasteurized milk and cottage cheese across the dairy value chain
- To assess dairy products availability, accessibility and physical distance access
- To assess knowledge status consumers on dairy products handling practices, safety and nutritional value

1.4 Significance of the study

The study was expected to generate valuable information on the dairy product value chain regarding the microbial safety and physicochemical properties of the dairy products (raw and pasteurized milk, and traditional and factory-made local cottage cheese). The study was anticipated to give an overview of the raw milk quality for milk processing plants. Accordingly, research findings may be expected to encourage a strong food system through inspiring policymakers, specifically on improving dairy sectors.

The study also expected to identify the critical points to take the interventions to improve dairy product safety and this helps the research and academic institutions for further research directions. But also, the current study gives a clue to conduct further research activities to identify the risk factors associated with poor microbial quality and practices which affects the physicochemical properties of milk and milk products. The study also generates valuable information on food environment drivers which affect the consumption of dairy products and safety.

2. Literature Review

2.1 Milk production System and Consumption habit in Ethiopia

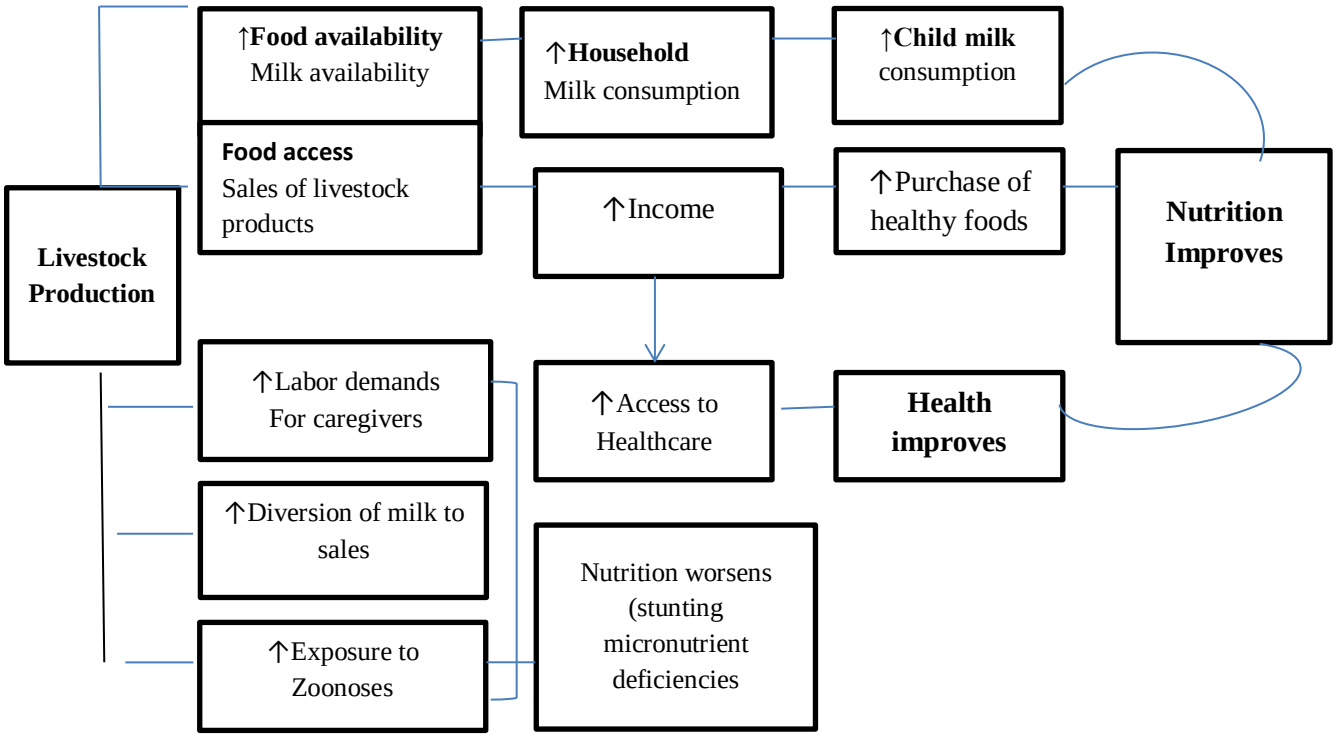
The milk production system can be categorized based on agro-ecology, socio-economic structures of the population, and type of breed and species. According to Getachew & Gashew (2011), milk production can be classified into two major systems, namely, rural dairy system (pastoralists, agro-pastoralists, and mixed crop-livestock producers) and, urban and peri-urban dairy systems. Ethiopia possesses the largest livestock population in Africa and the annual milk production is around 3.13 billion liters. The Oromia region is the leading region that produces 1.3 billion liters per year. SNNP is the second-largest milk-producing region with an estimated total of 725.8 million liters and followed by the Amhara region with 597 million liters and Tigray with 193 million liters (CSA, 2017). Ethiopia consumes approximately 19 kg/capita, although, the country has the highest livestock population in Africa. Approximately 83% of the total milk produced is consumed at the household level and only 7% is supplied to the formal and informal markets. The remaining balance is distributed between in-kind wages (0.43%) and used for processing local butter, yogurt, and cheese (10.06%) primarily as a means of extending the shelf life during times of surplus (Yilma, 2011).

2.2 Malnutrition of Children and the role of dairy products in the situation

The number of food-insecure people in Sub-Saharan Africa increased by more than 26 % and the prevalence of undernourishment increased by 0.3 % per year. Recent data show that in the developing world one of every four children under the age of five is still underweight and one of every three is stunted (Watson *et al.*, 2006). Currently, Ethiopia has made great strides in improving the nutritional and health status of children over recent decades. However, 38% of children under five remain stunted with 10% wasted, suffering from chronic and acute hunger and food insecurity. This varies greatly by region with 46% of under-five children stunted in Amhara and nearly 18% wasted in Afar (CSA, 2016). Animal source foods including meats, eggs, and dairy are an excellent source of protein, calories, and necessary micronutrients and have been linked to the improved nutritional status of children. Consumption of ASF is linked to improved cognitive function among undernourished children in low-income settings (Hop, 2003).

2.3 Nutrition-sensitive agriculture development: can dairying contribute?

Agriculture interventions aimed at improving nutrition, many rely upon income as a pathway to nutrition, or on increasing production that leads to greater food availability (World Bank, 2007). Much of agriculture-nutrition advocacy, however, cautions against over-reliance on income generation as a means to improve nutrition. Human nutrition is strongly related with agricultural activities and the food environment (food availability, accessibility, safety and nutritional quality and etc) depend on agricultural activities (Figure 1). Previous evaluations of agricultural interventions have shown that income may rise without improvements in nutritional status. Nutrition is not expected to be improved by income due to male dominance over the households and the linkage between agriculture and nutrition was not made and different nutrition fund programs preferring to invest in supplementation and food fortification (Von &Kennedy 1994; Smith *et al.*, 2003; World Bank 2007: FAO, 2013).



Source: Muelhoff *et al*. (2013)

Figure 1: The linkage between agriculture and human nutrition.

2.4 The concept of the dairy food environment

The food environment is the collective physical, economic, policy, socio-cultural surroundings, opportunities, and figure 2 indicates conditions that influence people's food choices and consumption (Swinburn *et al*., 2014; Gebru *et al.,* 2018). FAO (2016) defined the food environment as the interface that mediates one's food acquisition and consumption with the wider food system, encompassing dimensions such as the availability, accessibility, affordability, desirability, convenience, marketing, and properties of food sources. It includes also aspects such as food prices, composition, safety, labeling, promotion, the provision in schools and other settings, and food trade policies (Herforth & Ahmed, 2015).

2.4.1 Food Access

The concept of food access can be considered a proxy for food availability, which is the underlying causal mechanism hypothesized to affect residents' diets (e.g., more dairy products available in an area might lead to increased purchasing and consumption of dairy products by consumers, which may positively impact nutrition and health of them (Figure 2). It is determined by how well people can convert their various financial, political lands, and other assets into food, whether produced or purchased (Figure 2). The tremendous growth of urban areas has also encouraged a view of food security that emphasizes access and income (Ruel *et al.,* 1998). It reflects a geographical perspective of the food environment and includes measures such as proximity like distance to the nearest specified type of food outlet (Apparicio *et al.*, 2007).

2.4.2 Food availability

The FAO (2009) defined the availability of food as the amount of food that is present in a country or area through all forms of domestic production, imports, food stocks, and food aid. Food availability measures may quantify and characterize the actual foods available in an area and the amount of shelf-space dedicated to these items. The output from such availability measures often involves categorizing food environments as healthy or unhealthy based on the food outlets and specific foods available (Gustafson *et al.,* 2013). The food availability indicators capture not only the quantity but also the quality and diversity of food (Anderson & Elisabeth, 2015).

2.4.3 Food Quality and Utilization

Food quality is the quality of available food that influences food choices. But, the food utilization is determined based on nutritional value (daily requirements of calories, vitamins, protein, and micronutrients provided by the food consumed), social value, and food safety (White *et al.,* 2004). Determinants of nutritional value include a diversity of food consumed, type of primary protein, disease incidence (which affects food absorption), education, facilities for cooking and preparing food, access to clean water, and hygiene practices (Ericksen, 2007). Food utilization is affected by poor hygiene, food preferences, and the physiological condition affecting food absorption (Haddad & Gillespie, 2001; World Bank, 2006).

2.4.4 Food safety

Consuming unpacked food (contaminated food) which is later consumed by humans can result in food poisoning, sickness, or even death. Hence, maintaining hygiene during food handling is important to assure the safety of consumers as well as promote longer shelf-life of food products (Paine, 1992). Effective packaging contributes to reducing spoilage and maintaining food quality, although, several studies have also shown that packaging can be a potential source of food contamination (Muncke, 2009).

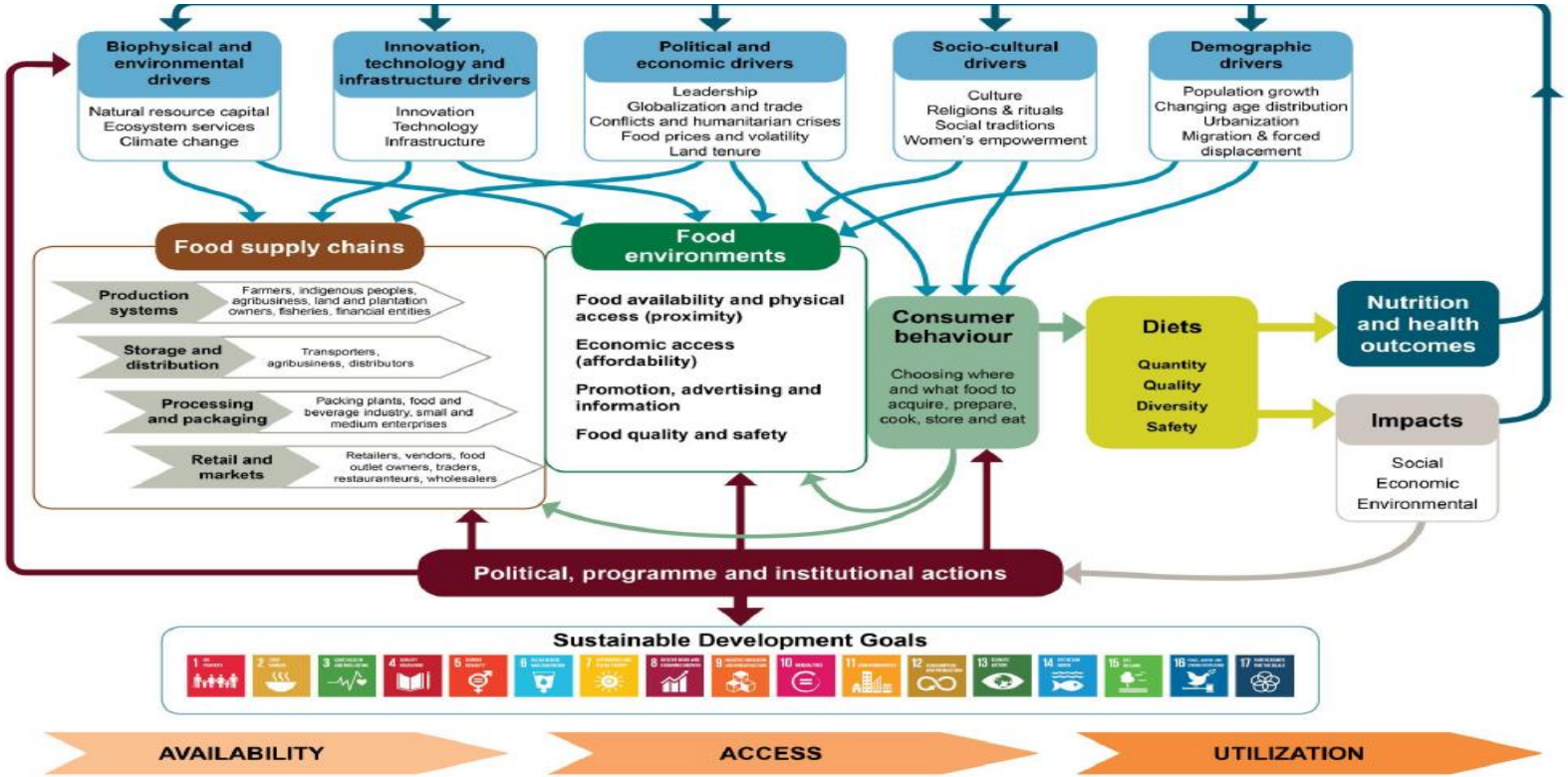
Quality upgrading responds to emerging consumer demand for improved dairy products, and thus depends on their World Trade Program (WTP) for higher quality products. For a broader upgrading strategy, access to reliable and trustworthy information regarding the nutritional value and health risks of dairy products may influence the purchasing behavior of larger groups of urban households. But it requires public investment in quality standards and private investments in processing infrastructure as well as improved packaging, labeling, and advertising (Jaffee *et al.,* 2011; Bekele, 2016a).

2.4.5 Food price

Affordability measurements consider the cost of foods within a defined area in either absolute, relative, or comparative terms. They have traditionally taken into account the cost of food relative to an individual's or household's purchasing power and aggregate food costs to an area level within a defined geographic area (FAO, 2009).

The food price in Ethiopia is not only high but also relatively more volatile. This abnormal food price surge put millions of rural and urban net food buyers at risk. In Ethiopia, a large proportion of household income (60%) goes for its purchase and a slight increase has great implications for the welfare of millions of households. With the increase in income, it is expected that consumption pattern shifts to high-value food items that demand encouraging the supply of livestock products (Tadesse, 2012).

Like other animal-source foods, dairy products tend to be an expensive source of energy compared with cereal staples. FAO (2009) indicated at times of economic stress livestock products are replaced by other proteins or starchy staples. In 2010, Land O'Lakes compared the reaction times as the top earners in Addis Ababa consumed about 38% of milk, while the lowest income group, approximately 61% of the population consumed only 23%.



Source: Gebru *et al.*, (2018)

Figure 2: Framework of food system for diets and nutrition

2.5 Dairy products: an excellent source of nutrition but expensive for the poor?

The greatest impediment to increasing the consumption of dairy products by the poor is their price. Like other animal-source foods, dairy products tend to be an expensive source of energy compared with cereal staples. However, the poorest rural families seldom keep dairy animals and for them, milk can be expensive and the poor urban families also have limited access to livestock products, including milk, because of their cost (FAO, 2011), where keeping animals allows them to diversify livelihood-generating activities and provides a source of locally produced food products for people living in the vicinity of the livestock keepers (Güendel, 2002).

The five pathways from agriculture to nutrition: subsistence-oriented production for household's consumption; income-oriented production for sale in markets; reduction in real food prices associated with increased agricultural production; empowerment of women as agents instrumental to household food security and health outcomes; and an indirect relationship between increasing agricultural productivity and nutrition outcomes through the agriculture sector's contribution to national income and macroeconomic growth (World Bank, 20007).

2.6 Common dairy products and nutritional value

2.6.1 Milk

Milk intake may be a marker for a nearly complete diet because of its high nutrient content (Fulgoni *et al.,* 2007). Milk fat contributes about half of the energy in whole milk and animal milk can play an important role in the diets of infants and young children in populations with a very low fat intake (Allen & Dror, 2011), where the availability of other animal-source foods (ASF) is limited. Milk proteins are synthesized from amino acids derived either from blood or synthesis in the epithelial cells. Approximately 80% of the proteins are caseins and 20% are whey proteins. The total protein content of the can ranges between 3.0 and 3.6% (Walstra & Jenness, 1984; Akers, 2002).

The most common carbohydrate in milk is lactose, which is specific to mammalian milk. The lactose content of milk is usually in the range of 4.6-4.8% and the lactose is synthesized in the Golgi apparatus and consists of galactose and glucose linked together (Akers, 2002). The range and mean of main constituents of cow milk are given in (Table 1). In addition to the main components of milk (water, fat, protein, and lactose), there are many other compounds present in milk. Milk contains numerous different components with a low molecular weight such as calcium, potassium, sodium, chloride, magnesium, zinc, phosphates, and citric acid. Milk is a major source of vitamin A and vitamin B in particular, but all vitamins are present in milk, although some in small concentrations e.g. vitamin C (Walstra & Jenness, 1984; Walstra *et al.*, 1999).

Table 1: Composition (%) of cow milk

Main constituent	Range (%)	Mean (%)
Water	85.5-89.5	87.0
Total solids	10.5-14.5	13.0
Fat	2.5-6.0	4.0
Proteins	2.9-5.0	3.4
Lactose	3.6-5.5	4.8
Minerals	0.6-0.9	0.8

Source: O'Connor, 1995

2.6.2 Arrera (defatted buttermilk)

The arrera is another byproduct of *ergo* obtained after the removal of *qibe, after* churning. It is either consumed in that form or cooked to produce *ayib*. In contrast to other traditional dairy products, *arrera* has fewer calories. It contains 91.5% moisture, 3.1% protein, 1.4% fat, 3.4% carbohydrate, and 0.6% ash. A hundred grams of '*arrera'* give 95 mg calcium, 84 mg phosphorus, 1.0 mg iron, 0.03 mg thiamine, 0.21 mg riboflavin, and 0.10 mg niacin (Kassaye *et al*., 1991).

2.6.3 Ititu (concentrated sour milk)

The name *ititu* is used for concentrated fermented milk prepared and consumed by the Borana tribes in southern Ethiopia. *Ititu* had an average pH of 3.65, titratable acidity (as lactic acid) of 1.92%, fat and protein content of 9.05% and 7.17%, respectively. *Ititu* had increased contents of free and total amino acids when compared to fresh whole milk and was rich in amino acids such as glutamic acid, alanine, proline, leucine, and serine. Based on a study on farm-made fermented milk in southern Ethiopia, reported that *ititu* had 3.3 - 3.7% fat, 3.3 - 3.6% protein, and 3.3 - 3.5% lactose (Kassaye *et al*., 1991).

2.6.4 Local cottage cheese (*ayib*)

Ayib is prepared after the manufacture of butter by heating the *arrera* to coagulate the curd and it is a soft curd-type cheese typical of many regions in Ethiopia and made from the buttermilk resulting from the churning of sour whole milk. The product is white but grainier than cottage cheese. It is very acidic but it is not stable enough for wide distribution and mainly consumed locally. *Ayib* is produced both at the home level, in small enterprises, and large scale plants owned by the state dairy enterprise. It is processed from fermented milk in all cases (O'Connor, 1995).

2.6.5 Augat (traditional whey)

This is the liquid part of *arerra* after the *ayib* is removed and it is rich in protein and free amino acids. *Augat* contains about 0.75% protein and can be consumed by humans or fed to animals (O'Connor & Tripathi, 1992).

2.7 Factors affect Chemical composition of milk

2.7.1 Breed and species

There are obvious differences in milk composition and yield among the various breeds of dairy cattle. Differences among individuals within a breed are often greater than differences among breeds (O'Connor, 1994) such differences are due to partly genetic factors and partly to environmental. For instance, Jersey breed gives milk of higher fat content than Friesian cattle, while Zebu cows can give milk containing up to 7 % fat (O'Mahony, 1998). The milk from indigenous cows contains 6.1 % fat, 3.3 % protein, 4.5 % lactose, and 0.7 % ash (Alganesh, 2002).

2.7.2 A feeding system and stage of lactation

According to O'Connor (1994), the ratio that increases milk production usually reduces the fat percentage of milk. The fat, lactose, and protein contents of milk vary according to the stage of lactation and the fat and SNF percentages tend to be higher in the early weeks of lactation, dropping by the third month then rising again as milk yield gradually declines (O' Manhony, 1998). The milk immediately after calving contains a very high percentage of total solids (up to 19 %) mainly due to the very high fat and milk protein contents (O' Connor, 1993).

2.7.3 Age and disease

The age of the cows has a slight but definite effect on the composition of their milk. As the cow grows older, the fat content of their milk decreases by about 0.02 percentage units per lactation while the fall in SNF is about 0.04 percentage units. The decrease in SNF content seems to be due to a decline in casein content. Both fat and SNF contents can be reduced by disease, particularly mastitis (O'Connor, 1994)

2.7.4 Adulteration practices

Middlemen attempt to counter the dilution by adding water, vegetable oil, starch, flour, sugarcane, whey powder, skim milk powder, and other ingredients to extend the solid

content of the milk (Fakhar *et al.*, 2006). In some cases, adding a small concentration adulterant like 0.2% of formalin may not have a significant influence on nutrition value rather a pH of the milk (Ayub *et al.*, 2007).

It is interesting to note that the middle-men attempt to counter the dilution by adding cane sugar to extend the solids content of the milk or as additives to mask the effect of dilution of water (Farkhar *et al*., 2006). While, the starch in milk is used to increase its viscosity or its profit margin and solid content of the milk (Afzai *et al.*,2011)

2.8 Heat-induced changes in milk and milk products

The production of heat-treated milk for human consumption covers the spectrum from pasteurization to in-container sterilization. Sakkas *et al.* (2014) suggested that the effects include degradation of lactose to organic acids and formation of lactulose, denaturation of whey proteins, destruction of vitamins and enzymes, hydrolysis of proteins and lipids, and disturbance of calcium/phosphorus equilibrium. Other effects include cooked flavor and nutritional value loss due to new substances formed by the Maillard reaction, which continues during the storage of heated milk (Elliott *et al.*, 2005).

In ultra-heat treatments (UHT), a preheating of the milk to 80 – 95°C can be performed to reduce whey proteins' ability to foul or deposit on the hot surfaces of the heat exchangers later (Villamiel *et al.*, 2009). Vitamins are sensitive to a different extent to heat, light, water activity, and the presence of oxidizing and reducing agents, especially, vitamin C and vitamin B are very sensitive to heat treatment. Losses of vitamin C in pasteurized milk have been reported to be as little as 0-10% but more severe pasteurization (e.g., 73°C for 10 min) results in about 26% losses (Mahindru, 2009).

2.9 Common Foodborne Pathogens in Dairy Products

Farm animals represent a major reservoir of pathogens that can be transferred to milk and common dairy products pathogens are *Staphylococcus aureus, Salmonella* spp., *Listeria monocytogenes, Escherichia coli* O157: H7, and *Campylobacter* spp. (Jakobsen *et al*., 2011). Most of the pathogens are the main microbiological hazards linked to raw milk and raw cheese (Van *et al.,* 2017).

2.9.1 *Salmonella* spp.

Salmonella spp. is gram-negative, motile, and facultative anaerobic bacteria that belong to the family *Enterobacteriaceae*. *Salmonella* infections can occur through the ingestion of contaminated food or water. *Salmonella* spp. can be transmitted to humans via the consumption of contaminated dairy products, especially unpasteurized or insufficiently pasteurized milk and cheeses, which cause outbreaks of *Salmonellosis* in human (Ahmed & Shimamoto, 2014). It is the most frequent cause of food-borne outbreaks, and human salmonellosis is the second most frequently reported zoonosis in the European Union (Wuyts *et al.,* 2013).

2.9.2 *Listeria* spp.

The genus *Listeria* spp. includes seven species, and these comprise *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. Ivanovii L. murrayi*, and *L. grayi*. These organisms can become endemic in food processing environments (McLauchlin *et al.*, 2004). *L. monocytogenes* is an intracellular Gram-positive non-sporulating pathogenic bacterium with a widespread presence in nature, affecting a wide range of domestic and wild animals and humans (Dowe *et al.,* 1997).

L. monocytogenes is one more example of a foodborne pathogen possibly contaminating raw milk and feces dirtying milking equipment are a source of contamination. Dairy products are identified as the main sources of listeriosis (Quero *et al.,* 2014; Jackson *et al.,* 2016). It can be present in raw milk and soft cheeses (Swaminathan & Gerner-Smidt, 2007) and it is of a significant public health concern as infection in pregnant women may

result in spontaneous abortions or stillbirth. Raw milk can be contaminated with *Listeria monocytogenes* from unclean equipment during milking, during storage in bulk tanks, or during transport to the cheese processing plant, where hygienic control measures may not be adequate (Melo *et al.,* 2015).

The threat is because it can grow and multiply during raw milk storage also at low temperatures (0-4°C) that implying that even the application of a correct cold chain would not eliminate the microorganism. That means an important factor in food-borne listeriosis is that the pathogen can multiply at refrigeration temperatures when given sufficient time (FAO & WHO, 2004). Because of its high case-fatality rate, listeriosis is, after salmonellosis, the second most frequent cause of foodborne infection-related deaths in Europe (Dalzini *et al*., 2016). However, outbreaks from *Listeria monocytogenes* are not common compared with those caused by pathogens such as *Salmonella* spp. (Todd &Notermans, 2011).

2.9.3 *Staphylococcus aureus*

S. aureus is a gram-positive bacterium, which causes mastitis in cows and other domestic dairy ruminants. It can contaminate milk via the teat canal, when there is an infection of the mammary gland, via the environment, or by bad hygiene habits during or after milking, such as, not washing hands when handling milk storage equipment. Foods of animal origin with high protein contents such as milk and dairy products, meat, meat products, salads, and bakery products favor the growth of bacteria, and this type of food has been frequently incriminated in *Staphylococcus aureus* outbreaks (Dhanashekar *et al*., 2012). This bacterium can grow in an extensive range of temperatures, pH values, sodium chloride concentrations, and water activity and it can also produce staphylococcal enterotoxins, which are responsible for staphylococcal food poisoning. *S. aureus* may cause diseases through the production of heat-stable enterotoxins. Later, it is very resistant to heating and pasteurization (Duquenne *et al.,* 2010).

2.9.4 Total coliforms and *E. coli*

In microbiological testing, total coliform is considered as an indicator organism or a marker that reflects the general microbiological condition of food or environment. For nearly a century, bacteria used in evaluating water for fecal contamination, and later in identifying unsanitary conditions in pasteurized dairy products and other foods. Coliforms are aerobic or facultative anaerobic, Gram-negative, non-spore-forming rods capable of fermenting lactose to produce gas and acid within 48 h at 32–35°C. Most bacterial genera that comprise the coliform group (e.g., *Escherichia*, *Klebsiella*, and *Serratia*) are within the family Enterobacteriaceae (Chapin *et al.*, 2014). The most pathogenic strains are referred to as verocyto-toxigenic *E. coli* (VTEC), Shiga toxin-producing *E. coli* (STEC), and enterohemorrhagic *E. coli* (EHEC), also known as *E. coli* serotype *O157: H7*. Cattle feces are the major reservoir of EHEC which commonly contaminates bulk tank milk. Milk contamination is hence a result of direct exposure to fecal material or environmental contamination and raw milk poses a risk for STEC, and several outbreaks have been recently reported for this pathogen (Van *et al.*, 2017).

2.10 Detection methods and characterization of foodborne pathogens in dairy products

2.10.1 Cultural method

Traditional methods for the detection of bacterial pathogens in foods have been widely used because they are sensitive and inexpensive and can give both qualitative and quantitative information on the number and the nature of the microorganisms present in the food sample These methods are standard methods; however, they are extremely laborious, time-consuming (requiring several days), and often inconclusive (Zhao *et al*., 2016). The conventional methods for the detection of these pathogens involve identification and confirmation based on culturing on selective media along with biochemical tests and immunological assays. Detection usually involves the following three steps; non-selective enrichment, selective enrichment and isolation, and identification (Arqués *et al*., 2015; Quigley *et al.*, 2013).

2.10.2 Microbial Characterization

The morphological and biochemical method of identification of bacteria is the classical method of characterization of bacteria. Classical identification of individual bacterial species in environmental samples typically involves isolation, laboratory culture, and then taxonomic characterization. The classification of bacteria into families, genera, and species is based on a wide range of phenotypic characteristics. The conventional methods include phenotypic characterization (colony morphologies, Gram staining, endospore staining, etc.), biochemical characteristics (nutrient requirements - sugars, enzymatic activities, and /or metabolic activities). But in conventional methods, the characteristics are not static and can change with stress or evolution (Chiang *et al.*, 2012).

Microbial fermentation is a biochemical activity that is carried out by microbes that convert organic macromolecular compounds into simpler compounds in anaerobic conditions. It can produce a variety of end compounds, such as carbohydrate fermentation to produce various acidic compounds such as lactic acid and propionic, esters, ketones, and gas. Most of the microorganisms obtain energy from carbohydrates in the form of the further substrate in fermentation produces organic acids (such as lactic acid, formic, acetic), accompanied or not accompanied by the formation of gas. The medium contains different pH indicators for color-changing (MacFaddin, 2000). For example, methyl red is a test used to identify mixed acid fermenting bacteria that yield a stable acid end product causing the pH to drop below 4.4. Positive results are characterized by the color changes to red after adding methyl red (Holbrook & Anderson, 1980).

Simmon's Citrate test is used to look at the ability of enteric organisms based on the ability to ferment citrate as a carbon source. Citrate is a selective test used to help differentiate species of the family *Enterobacteriaceae* media and is utilized as a single carbon and nitrogen source. To test this ability; bacteria are incubated in a medium that contains only citrate as a source of carbon and ammonium phosphate as a nitrogen source. The medium contains a bromothymol blue indicator that will turn blue at the positive reaction and remain green if negative reactions. Bromothymol blue is used as an indicator when the citric acid is metabolized, carbon dioxide is generated which

combines with sodium and water to form sodium carbonate which is an alkaline product that is responsible for the change in color from green to blue (Barrow, 1993).

The indole test is used to see the ability of bacteria to degrade amino acid tryptophan enzymatically. This test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. Tryptophan is an essential amino acid, which is oxidized by some bacteria resulting in the formation of indole, pynivic acid, and ammonia.The indole test is done by inoculating the test organism into tryptophan broth, which contains tryptophan and bacteria to use an enzyme, tryptophanase to break down the amino acid, tryptophan, which makes by-products, of which, indole is one. The indole which is produced is detected by adding KOVAC's reagent which produced a cherry red-colored ring (MacFaddin, 2000; ISO, 6888-1:1999).

Triplate sugar iron (TSI) agar is used to test the ability of microbes in sugar fermentation and hydrogen sulfide production. TSI agar consists of glucose, sucrose, lactose, pH indicator phenol red, and ferrous sulfate. The sugar fermentation products result in an acidic environment that will turn both the butt and slant yellow. If hydrogen sulfide is produced, it will react with the iron in the agar to form ferrous sulfide, which can be observed as a black precipitate in the butt (Barrow, 1993; MacFaddin, 2000).

2.10.3 Molecular genetics method

Cultural methods are now giving way to molecular diagnostic methods based on DNA analysis, such as polymerase chain reaction (PCR), multiplex PCR, and real-time quantitative PCR (qPCR), which have been used for rapid and reliable detection of foodborne pathogens (Chiang *et al.,* 2012). Besides, typifying methods are also largely used for accurate genetic characterization in outbreak investigations techniques applied to dairy products to detect and identify foodborne bacteria. Molecular techniques for pathogens are being developed for various aspects of detection, such as sensitivity, rapidity, and selectivity (Zhao *et al*., 2014).

2.11 Milk Quality tests

There are four tests for milk quality such as; organoleptic, clot-on-boiling, alcohol, and lactometer test. These tests are routinely carried out at milk collection points to ensure that only milk of acceptable quality is received (Lore *et al*., 2006).

Lactometer test serves as a quick method to determine adulteration of milk by water and the organoleptic test is done through smelling and taste of the milk (Pandey & Voskuil, 2011). This test is based on the density of whole milk which ranges from 1.026 to 1.032 g/ml. Therefore, adding water to milk lowers its density, while the addition of solids increases the density of milk. However, boiling clot and alcohol tests are a quick method for test raw coming milk for further heat processing (Kurwijila, 2006).

2.11.1 Clot on boiling test

When milk developed too much acid or pH value less than 5.8, it becomes abnormal for further processing. The simple and quick method to test abnormality is a clot-on boiling test and most of the time it is performed by applying heat to boil on a spoon, test tube, or any suitable container (O'Connor, 1995). The sensitivity of the test is varied based on the sourness of the milk. When the milk coagulates or precipitates, the milk fails the test (Draaiyer *et al*, 2009).

2.11.2 Alcohol test

An alcohol test is also a quick method and used as a screening test for milk processing. It is established on the instability of the protein mainly casein when the concentration of acid or rennet is increased, levels of albumen (colostrum milk), whey protein, and salt concentrate result in a positive test. The test is done by mixing an equal volume of alcohol and milk samples in small volume test bottles or test tubes. If a negative result is obtained (no coagulation, clotting, or precipitation upon shaking, the milk samples has good quality (O'Connor, 1995; Draaiyer *et al*, 2009). The alcohol test can detect milk whose pH is 6.4 or lower and is more sensitive than the clot on- boiling test, which only detects milk pH levels of 5.8 and below (Kurwijila, 2006).

2.12 Sources of microbial contamination of dairy products

2.12.1 Milking practices and equipment

Poor milking practices may lead to contamination of raw milk. The teat surface is the major avenue of entry of micro-organisms into raw milk. It is well recognized that there is a significant opportunity for teats to become contaminated by feces and soil (Cook & Sandeman 2000; Vaerewijck *et al.*, 2001). Milk residues left on equipment and utensil surfaces provide nutrients to support the growth of many microorganisms, including pathogens. In the case of cracked milking equipment, a large number of bacteria enter and grow in the cracks, which are difficult to clean (Bramley & McKinnon, 1990; Bryan, 1983). The bacterial load of milk increases during transportation and if the transportation equipment is not appropriate the bacterial counts increase causing spoilage before milk reaches its destination (Grillet *et al.*, 2007).

2.12.2 Environment and seasons

The environment is also a major source of microorganisms on the dairy farm. It was found that milking parlor air and bird droppings (were major contamination sources during winter, while feeds water, calf bedding, soils, milking parlor air, and bird droppings were the main culprits in the spring. All animal and environmental samples except milking parlor air and bulk tank milk were found to contribute significantly to the presence of bacteria in the summer; whereas the major sources of contamination were feeds, cow bedding, cow soils, air, and insects during the fall (Pangloli *et al.*, 2008).

2.12.3 Contaminated water and personal hygiene of the milker

Water used in the production of milk should be of potable quality. But also, the storage tanks should be protected to prevent access by insects, rodents, birds, and other sources of contamination, and equipment used to deliver water should be properly cleaned. However, the problems may arise when untreated water supplies are used to rinse and wash equipment. Such water may contain a diverse array of microorganisms including

Pseudomonas spp., coliforms, *Bacillus* spp. and numerous other types of bacteria (Bramley & Mckinnon, 2004).

Indeed, Perkins *et al.* (2007) have demonstrated the potential for contamination of milk with *E. coli* through the wash water. The number of cells contaminating the milk may be small but there is the potential for growth in any residual water remaining on the equipment. Milk handling personnel (milker) may contribute various organisms including pathogens especially when they are careless, uninformed, or willfully negligent, directly to milk. Organisms may drop from hands, clothing, nose, and mouth and sneezing and coughing. Milking men need to be in good health so that they can be a source of infectious diseases such as tuberculosis (Kurwijila, 1998).

2.12.4 Animal Health

Animal health is an essential part of milk production as it influences the quantity, safety, and quality of milk being produced (Quinn *et al.*, 1994). Therefore, the health status of a dairy herd is the first indicator of the safety and quality of milk and dairy products. Furthermore, unhealthy animals, particularly lactating cows can produce unsafe milk due to the shedding of microorganisms that can cause infection in both animals and humans. Hence, poor animal health can have a negative public health impact by increasing the risk of foodborne illness (Quinn *et al.*, 1994; Radostits *et al*, 1994; Godefay & Bayelegn, 2000; Jay, 2000; Alehegne, 2004). Bovine tuberculosis caused by *Mycobacterium bovis* and brucellosis caused by *Brucella* is among the major animal diseases that can impact public health through the consumption of raw milk produced by infected herds (Acha & Szyfres, 2003).

2.13 Control Measures of Microbial Contamination in Raw Milk

2.13.1 Cooling

To prevent or retard the growth of bacteria in milk and to maintain its quality for domestic consumption or during transport to the processing plant, it is essential to cool the fresh milk as quickly as possible (O'Connor, 1995). To facilitate the bulking of raw milk supply and transport the incoming milk, refrigeration facilities are provided at points of collection and transport means to maintain the temperature as much as possible (Getachew *et al.,* 2008).

In the tropical countries of Africa with high ambient temperatures, lack of refrigeration facilities at the farm and household level imply that raw milk will acidify very fast. Therefore, the collection systems must be designed to move the milk to the cooling and/or processing center in the shortest possible time. Also, every effort should be made to use available systems such as water cooling, air circulation, or shaded areas to reduce milk temperature (Godefay & Bayelegn, 2000)

2.13.2 Boiling

It is the easiest and most practicable method of making milk safe in every home. Boiling involves raising the temperature to the boiling point and maintaining at this temperature for a few minutes. Then, the milk should be cooled immediately and the temperature maintained below 10°C. Since this may be impracticable at home, preferably the milk must be consumed as soon as possible after cooling and not an extended period after it has been boiled and cooled (Linton, 1982; Gebra-Emanuel, 1997).

2.13.3 Pasteurization

The Codex Alimentarius (2004) defined pasteurization as microbiocidal heat treatment aimed at reducing the number of any pathogenic microorganisms in milk and liquid milk products, if present, to a level at which they do not constitute a significant health hazard. Based on the temperature and the time applied, pasteurization can be classified as HTST (high-temperature short-time) and LTLT (low-temperature long-time) pasteurization.

The former is also referred to as low pasteurization and the condition commonly used for milk is 72°C for 15s, whereas the LTLT pasteurization is performed at 63°C for 30min or at 68°C for 10 min. Heating conditions (temperature and time) depend on the raw milk microbiological quality, on milk fat or sugar content, and also vary from country to country based on microorganism strain heat resistance. Thus, pasteurization can be performed also at temperatures higher than 85°C for the 30s (high pasteurization). The increase in temperature and/or an extension of holding time is recommended to inactivate heat-resistant strains of *L. monocytogenes, E. coli, and Campylobacter* spp. (Kelly & O'shea, 2011). The best keeping quality of pasteurized milk is achieved by using temperatures below 77°C that do not inactivate the lactoperoxidase enzyme (LPO) and do not stimulate the growth of spores (Driehuis, 2014).

2.13.4 UHT of milk processing

Sterilization like UHT processing aims to produce a product that has a shelf life between 6-12 months at room temperature and remains unchanged in this period even if it usually is consumed earlier (Deeth, 2010). The UHT treatment is usually carried out at temperatures between 135 – 150°C for 1 – 10 seconds to achieve commercial sterility. The UHT processing of milk destroys all microorganisms that can grow under normal storage conditions (Villamiel *et al.,* 2009). Almost all enzymes are also inactivated by UHT processing due to most enzymes in milk are inactivated at temperatures below 100°C but some proteinases and lipases need temperatures above 150 °C for inactivation (Kessler, 2002).

2.13.5 Thermization

Thermization is a heat treatment usually performed at 60 to 69°C for the 20s. The main purpose is to kill bacteria, especially psychrotrophics, thus preventing the production of heat-resistant lipases and proteinases that may impair the milk keeping quality. Thermization thus enables to extend the storage time of raw milk before processing and to enhance the keeping quality of milk (Sun, 2012). Nevertheless, it does not ensure milk safety, as it cannot eliminate pathogens like *L.monocytogenes* can grow in chilled-stored thermized milk (Fernandez, 2009).

2.13.6. In-Bottle Sterilization

In-bottle sterilization is commonly performed at 110°C for 30 min. However, the temperatures ranging from 105°C to 120°C for 20–40 min can be used. All pathogens and non-pathogens microorganisms are destroyed, as well as spores. A 9-log reduction in the spores of thermophilic bacteria and 12-log reduction of *C. botulinum* are obtained. All milk enzymes are inactivated but not all bacterial lipases and proteinases. It has also a detrimental effect on the organoleptic and nutritional quality of milk (Ustunol *et al*., 2014).

2.13.7 Microfiltration

Microfiltration (MF) is a non-thermal treatment method with the specific advantage of being very effective in the removal of bacterial spores in comparison with conventional pasteurization. But, the major drawback of MF is fouling at the membrane surface which adversely affects selectivity and requires frequent rinsing and cleaning procedures which can have a detrimental effect on the cost-effectiveness of the technology. It offers several opportunities to the dairy chain, as it allows milk products to keep organoleptic characteristics which are similar to fresh milk with improved shelf-life. A good number of micro-filtered milk is available on the market and its success is due to a perceived freshness and the above-mentioned extended shelf-life (Tomasula *et al.,* 2015).

2.13.8 Ultrasound

Ultrasounds are waves with a frequency higher than 20 kHz, with a distinction between low and high-intensity ultrasounds, which have a power level of 0.1 MHz and 10–1000 W cm^{-2}, respectively. In ultrasonic treatments, ultrasound waves travel through a liquid, alternating compression and expansion cycles. During the expansion cycle, high-intensity ultrasound causes the growth of existing bubbles which implode violently when they attain a volume at which they do not absorb more energy. At the implosion phase, locally very high temperatures (up to 5500°C) and pressures (50 MPa) are reached inside the bubbles and this has a detrimental effect on microorganisms. The main applications of ultrasound in milk and dairy products are due to its effect in inactivating bacteria and enzymes, homogenizing milk, extracting enzymes, and lactose hydrolysis. However, it has been stated that the energy consumption required in ultrasound application to kill microorganisms is higher than for conventional methods. Moreover, it has been demonstrated that ultrasound on its own is not very effective for the inactivation of microorganisms and enzymes in milk; combinations of ultrasound with heat (thermos sonication) and pressure (mano-sonication) have been developed (Zisu & Chandrapala, 2015).

3. Materials and Methods

3.1 Description of the study areas

The study areas are located in the Oromia region which is found in the central high land of Ethiopia and the region is dominantly known for its high milk production. The areas also experience a bimodal rainfall pattern with a long rainy season extending from July to September, while the short rainy season extends from March to April. The area is characterized by mild subtropical weather, with an average minimum and maximum annual temperatures of 6.3°C and 22.1°C, respectively. Chancho is located 45 kilometers northwest of Addis Ababa.

The altitude is between 2500 and 2600 meters above sea level. The average daily temperature is about 15°C with a minimum of 10°C and a maximum of 23°C and the average rainfall varies between 800-1200 mm per year. Wolmera is located forty kilometers north-west of Addis Ababa at an altitude of 2400 meters above sea level. The average minimum and maximum temperature in this area vary from 6 to 22°C and the average yearly rainfall is 1100 mm (CSA, 2004; Vernooiji *et al.*, 2010).

3.2 Design of the Study

The design of the study was undertaken as a cross-sectional study design to assess microbial quality and physicochemical properties of the milk and cottage cheese across the dairy value chain. Milk and cottage cheese (*ayib*) producers, union and collection points, and raw milk retailers were identified at Womera and Chancho study sites. However, other dairy value chains like pasteurized milk and cottage cheese retailers, and dairy products consumers were identified in Addis Ababa city due to its closeness to two of the study areas and dairy products market supply. A mixed-method sampling method (probability and non-probability sampling) was applied for the overall study. Then, a total of 65 dairy product samples were collected across the dairy value chain.

A total of 40 raw milk samples from producers, collectors, retailers, and union gates (10 samples from each point) and 10 pasteurized milk from the retailers, and 15 cottage cheese from farm markets and retailers (10 samples from two farm markets and five samples from the retailers). A total of 120 dairy products consumers were identified in the purposive selection method in Addis Ababa city from the sub-cities (10 respondents from each of the sub-cities). The respondents interviewed face-face to identify the dairy food environment which can affect dairy product consumption.

3.3 Sampling techniques and Handling Practices

The sampling was done according to the general guidelines set by the International Organization for Standards (ISO, 707: 2008). The plastic bottles were cleaned and autoclaved before sampling according to general guidelines provided for the sampling of the microbial analysis. Samples taken from the bulk of the milk was agitated by a locally available dipper called large spoon (‘*chelfa’*) prior sample taking. The material was washed thoroughly with autoclaved distilled water and disinfectant (70% alcohol) and finally rinsed with autoclaved distilled water, and the practices were repeated between consecutive sampling. During the cottage cheese sampling from farmers’ markets, the spoon was inserted in alcohol and allowed to flame, then cool by inserting in autoclaved distilled water and the practices were repeated between the samples.

For physicochemical and microbial analysis, 200 ml/gram of the milk samples and cottage cheese were aseptically collected. In addition, nearly 500 ml/gram for milk and cottage cheese samples collected separately for mineral analysis. Then, the samples were labeled and placed in ice packing and transported to Holetta Dairy Research Laboratory. These samples were transferred into the refrigerator immediately at 4°C and culturing was conducted within 24-48 hrs.

3.4 Quality tests of milk

3.4.1 Clot on boiling test

The clot-on boiling test of the milk was performed according to the method described by Marshall (1992). A test was done immediately after the samples were delivered to the laboratory within a few hours. A spoon of the milk was taken and allowed on a Bunsen burner for nearly five minutes. Then, milk samples turned to curd or clot during the heat treatment was taken as a positive result, while the samples which unclotted were considered as a negative result.

3.4.2 Alcohol perception test

The alcohol test of the milk samples was done according to the standard method provided by Marshall (1992). A 2 ml of the raw milk sample was and mixed with an equal volume of 68% ethanol solution in a sterile test tube. Then, the mixture was agitated and coagulated samples were taken as a positive result while unclotted samples were taken as a negative result.

3.5.3 pH and Acidity

The Acidity and pH of the milk and cottage cheese samples were done according to the method described by O'Connor (1995). The pH meter device was calibrated or standardized with two standard buffer solutions at 4 and 6.68 before actual sample measurement. Then, 10 ml of fluid milk sample was taken by measuring pipette and the probes put into the samples and the reading was taken after the reading became stable. Unlike milk, 10 grams of the cottage cheese sample was weighed and dissolved in 100 ml of the distilled water and further dissolved by a magnetic stirrer. Thereafter, the solution of the sample was filtered by Whatman paper and 10 ml of the supernatant of the solution was taken and kept for a pH meter reading.

The titratable acidity of the milk and cottage cheese was performed by titration method using a standardized concentration (0.1N) of the sodium hydroxide solution.

Then, 10ml of milk and cottage cheese samples were titrated by 0.1N of sodium hydroxide solution until faint pink color persisted. Finally, the acidity of the sample was expressed as a percentage of lactic acid as shown below

$$Acidity\ or\ Lactic\ acid\ (\%) = \frac{ml\ of \frac{N}{10}\ Alkali \times 0.009 \times 100}{ml\ of\ the\ sample}$$

3.5 Proximate Composition Analysis

3.5.1 Total Solid

Total solid portions of the milk and cottage cheese were determined by the oven drying method according to standard procedures provided by Michael & Joseph (2004). Duplicate of 5 ml/gram milk and cottage cheese samples were homogenized and weighed in pre-dried and weighed crucible dishes. Then, the samples were kept in a water bath containing boiling water for one hour, and finally, the samples were allowed to dry in an oven at 105°C for 24 hours. Then, the dried portions of the samples were cooled and reweighed and the percentages of the total solid were calculated using the following formula:

$$Total\ Solids\ (TS\%) = \frac{Weight\ of\ dried\ samplle}{Sample\ weight} * 100$$

3.5.2 Fat content

The fat content of the cottage cheese and fluid milk samples were determined according to standard procedures (Richardson, 1985 & Michael and Joseph, 2004). A 3 gram of cottage cheese samples were weighed and transferred to a dried butryometer in duplicates and 10 ml of sulfuric acid (90% con.) was added to the samples. Then, 3 ml of distilled water was first added to each butryometers and 4 ml of distilled water additionally added to homogenize the sample portion and one ml of amyl alcohol was added to butryometer. Unlike cottage cheese, 11 ml of fluid milk was added to butryometer using milk pippete having sulfuric acid (90% con.) similarly, one ml of amyl alcohol was added, and then, the stopper of the butryometer was put on and samples were shaken and inverted several

times until the samples completely digested by acid. Then, butryometer was kept in a water bath for 5 minutes at 65℃ and centrifuged in Gerbercentrifuge for 5 minutes at 1100 rpm (rotation per minute).The butryometers were placed again in a water bath at 65℃ for 5 minutes and the percentage of the fat was recorded directly from the butryometer reading.

3.5.3 Protein Content

The protein content of the fluid milk was determined according to procedures provided by O'Connor (1994). A 10 ml of milk sample was added to a beaker; consequently, 0.4 ml of 0.4% of potassium oxalate was added to a beaker. Then, 0.5 ml of 0.5% phenolphthalein indicator added to beaker containing milk samples and potassium oxalate. Thereafter, the sample was titrated by 0.1N of NaOH using the digital burette, and the consumed volume of the sodium hydroxide was recorded. Finally, the percentage of protein of milk samples was calculated by multiplying the volume of sodium hydroxide consumed by 1.74 conversion factors (Foley *et al.*, 1974).

3.5.4 Ash content

The dried cottage cheese (*ayib*) and fluid milk samples leftover of the total solid were used for the determination of the ash percentage of the samples. Then, samples were ignited in a Muffle Furnace at 550°C for 5 hours, until the ash was becoming carbon-free. The samples were then placed in desiccators for cooling and re-weighed. The initial and final weight was of the samples was taken which was considered as the weight of the residue and it was divided by the original sample weight to calculate the ash value of the samples. Accordingly, the percentage of the ash content was calculated using the following formula Michael and Joseph (2004).

$$Percentage\ of\ Ash\ = \frac{Weight\ of\ the\ residue}{Weight\ of\ the\ sample} * 100$$

3.5.5 Lactose Content/Carbohydrate

The percentage of the lactose was calculated based on obtained values (total solid, fat, protein, and ash) by difference O'Mahonny (1998).

$$Percentage\ of\ Lactose = Percentages\ of\ (Total\ solid - Fat - Protein - Ash)$$

3.5.6 Solid non-Fat content (SNF)

The SNF content of the samples was determined by subtracting the % of fat from the total solid value for milk and cottage cheese O'Mahony (1998).

$$Percentage\ of\ SNF = Percentages\ of\ (Total\ Solid - Fat)$$

3.6 Mineral Analysis

3.6.1 Phosphorus analysis

The phosphors content of milk and *ayib* was determined using a UV-Vis spectrophotometer at 690 nm according to the standard method (Latimer, 2016). One gram of dried milk and cottage cheese was ashed using a muffle furnace at 550 ^{0}C for 4 hrs. The ash was dissolved in 5 ml of 6 M HCl. Subsequently, 15 ml of 3 M HCl was added and heated until the solution boils. The digested sample was cooled, filtered, and adjusted to the required volume using demineralized water. The instrument was calibrated before sample analysis by potassium dihydrogen phosphate (KH_2PO_4) as phosphorus standards (0, 2, 4, 6, 8, and 10) ppm. Then, the phosphorus content of the samples was calculated as shown below:

$$\text{Phosphorus}(\%) = \frac{(\text{R} - \text{b}) * \text{V} * \text{Df}}{\text{S} * \text{Aliq}}$$

Where R- reading (absorbance of phosphors in ppm)
b- Blank sample
V- Total volume
DF – dilution factor
S – Sample weight of dried milk and cheese
Aliq – aliquot of the sample taken for the reading

3.6.2 Calcium, Zinc, and Iron analysis

The mineral analysis of dried milk and cottage cheese such as Calcium, Iron, and zinc was carried out using (Agilent, USA) model AA 4200 flame atomic absorption spectrophotometer equipped with Spectra AA (Agilent, Australia) hollow cathode lamps as the radiation source (Ieggli *et al*., 2010). Acetylene–air flame was used as an energy source. The gas flow rates and the burner height were adjusted to obtain the maximum absorbance signal for each element.

$$Mineral\ Content\left(\frac{mg}{100\ g}\right) = \frac{(R-b)*V*df}{10*Swt}$$

Where: R – Reading (Concentration of minerals in ppm)
B – Blank sample (without milk and cheese samples)
V- Total volume
DF – dilution factor
S – Dried milk and cheese samples 1g

3.7 Microbial Analysis

3.7.1 Isolation of *Salmonella* spp.

Isolation of *Salmonella* spp. from milk and cottage cheese was based on the recommendation by the International Standard Organization (ISO 6785:2002). A 25 ml/g of fluid milk and cottage cheese were taken by sterile measuring pipette and weighing boot, respectively. Isolation of *Salmonella* spp. involved three basic stages. The first stage was pre-enrichment of *Salmonella* spp. in samples, by mixing 25 ml/gram in 225 ml of sterilized buffer peptone water and allowed to incubate for 24 hours at 37℃. The second stage was an enrichment of *Salmonella* spp. in which 0.1 ml of pre-enriched culture was transferred into 10 ml sterilized selective broth, Rapport Vassiliadis soy peptone (RVS), and incubated at 41.5±0.5℃ for 18-24 hours. The final stage was culturing *Salmonella* spp. through a loop of the samples was taken from selective medium and streaked onto Xylose Lysine Deoxycholate (XLD) agar plates and further incubated at 37℃ for 24-48 hours.

Morphologically, the presumptive colonies of *Salmonella* spp. on XLD agar which was a pink colony with or without black center or glossy black centers were selected. The morphological identification was assisted by American Type Collection Culture (*Salmonella typhimurium ATCC 14802*). Then, the presumptive colonies were sub-cultured on Brain Heart Infusion (BHI) agar for further biochemical tests (Table 9).

3.7.2 Isolation *Listeria* spp.

Isolation of *Listeria* spp. from milk and cottage cheese was done according to the standard protocol recommended by the International Organization for Standards (ISO 11290-1:2004). Three basic stages were involved to isolate *Listeria* spp. from the samples and bacteriological media used at the different stages was prepared according to the manufacturer's recommendation. The reference strain, *Listeria monocytogenes* (*ATCC 15313*) was used as positive control along the stages of the *Listeria* spp. isolation from the milk and cottage cheese. The pre-enrichment of the samples was done by adding 25ml/gram of the milk or cottage cheese to 225ml of the sterilized buffered peptone water. Then, the mixture was mixed well and allowed for incubation at 25℃ for 24 hours. Then, 1 ml of pre-enriched culture was taken and added to 9 ml of the sterilized Listeria enrichment broth (Twin pack A and B mixture) which supplemented by naladixic acid at the secondary stages of *Listeria* spp. enrichment, and further incubated at 37℃ for 48 hours. After the successive enrichment stages, 0.1ml of selective enrichment broth was taken and plated on PALCAM agars (supplemented by polymycin B, ceftadizime, acraflavine hydrochloride antibiotics), and incubated at 37℃ for 48 hours.

Morphologically, the presumptive of *Listeria* spp. colonies on PALCAM agars which were black with a halo the center were identified. The selection was assisted by the reference strain, *Listeria monocytogenes (ATCC 15313*). Then, the colonies were transferred to Brain Heart Infusion agar (BHI) and allowed 48 hours incubation. Finally, a series of biochemical tests were conducted for the final confirmation result (Orsi & Weidmann, 2016).

3.7.3 Isolation of *Staphylococcus aureus*

Isolation of *Staphylococcus aureus* from the milk and cottage cheese was done by spreading according to the standard method (BAM, 2003). A 25 ml/g of the fluid milk or cottage cheese samples were measured aseptically and added to 225 ml of the sterilized buffered peptone water (ratio of 1:9 volumes), then, the mixture was homogenized gently by vortex mixer, and the serial dilutions were done from 10^{-1} to 10^{-6}. A 0.1 ml of the sample suspensions were transferred to baired parker agar plates and inoculated over the surface of the agar plate, using a sterile bent glass rod. The plates waited until the inoculum was absorbed by agar and incubated for 48 hours at 35℃. A loop of the reference strain (*Staphylococcus aureus ATCC-25923*) was streaked over the surface of agar as a positive control of the experiment. The presumptive colonies which were circular, smooth, moist, gray to jet-black, and colonies with buttery to gummy consistency when touched with inoculating needle were identified. But, the colonies which were catalase positive were considered as *Staphylococcus aureus* by catalase test. But also further biochemical tests were done for confirmation of the organism (Table 9). Finally, the counting between 20-200 colonies were selected among dilutions and expressed as CFU/ml or gram for fluid milk and cottage cheese.

3.7.4 Total coliform count

Enumeration of total coliform in fluid milk and cottage cheese was done by pour method according to standard procedures recommended by (Marshaa, 1992). Accordingly, 25ml/gram of milk and cottage cheese samples were diluted in 225ml of sterilized peptone water for initial dilution. Then, one ml of the initial dilution was taken and added into a sterile test tube having 9 ml of peptone water. After mixing, the sample is serially diluted up to 10^{-5} and 1 ml of inoculum was taken from all dilutions and mixed thoroughly with molten 15–20 ml Violet Red Bile Agar. After thoroughly mixing, the plated samples were allowed to solidify and then incubated at 37 °C for 24 hours. Typical dark red colonies are considered as coliform colonies and counted by the digital colony counter.

Approximately, the plate contained the number of colonies between 30 and 300 was selected among the dilutions and finally, the CFU/ml or g of the total coliform count in the samples was done using the formula given by Marshal (1992).

3.8 Biochemical Identification

3.8.1 Gram staining

Gram staining was done to determine Gram-positive or Gram negative. A drop of distilled water was put on glass slide and isolated colony was taken, and mixed with water. The mixing was continued till become turbid and allowed for air dry and heat, finally allowed to cool. Crystal violet was flooded over the slide, then washed by running water, and then by Gram's iodine. After, Gram's iodine was washed by running water; it was rinsed by alcohol (95%) for 5-10 seconds. Then, it was rinsed by running tap water and counter stain with safranin for nearly one minute (60 seconds), finally rinsed by running tap water and allowed to dry. The cell of bacteria decolorized by alcohol and took red to pink color during counterstaining with safranin was taken as Gram negative, while the cells retained the crystal violet and remained purple to dark blue color was taken as Gram positive (MacFaddin, 2000).

3.8.2 Motility test

The test was done to identify whether isolate was motile by means of flagella or not. The medium (motility test medium) was stabbed with small amount of the inoculum. Then, the tube was incubated overnight at room temperature. When grow of isolate was radiated from the line of inoculum, it was taken as motile (Winn *et al.,* 2006).

3.8.3 Triple Sugar Iron (Oxoid) Slant

The medium was used to test utilization of specific carbohydrate (glucose, lactose and sucrose) and production of gas along with or without hydrogen sulfide production (H_2S). The procedure followed MacFaddin (2000) and the Triple Iron Agar was prepared according to the manufactures instruction. Then, 10 ml of the molten agar was added to test tubes and slanted. After the agar was solidified, the butt was stabbed and the slant

was streaked to detect the fermentation of glucose, lactose, sucrose as well as production of H_2S gas. Then, the slants were incubated at 24℃ for 18-24 hours (Table 9).

3.8.4 Simmon's Citrate Agar

The test was done to identify organisms were able to use the citrate source of carbohydrate and able to grow on the agar (Barrow, 1993).The agar was prepared according to the manufactures instruction. The isolate was streaked on slat agar and incubated at 24℃ up to four days. The positive result was recorded when the medium changed from green to blue color (Table 9).

3.8.5 Endospore test

Endospores test is carried out to determine the ability of bacteria to produce endospores. The fixed preparations are placed on a water bath then covered with filter paper. Green malachite drops are dropped and left for 5 minutes. The preparation is then washed with running water. Then re-color with safranin then leave for 60 seconds. The preparation was washed and then observed under a microscope. When the endospores were green while vegetative cells are red, it was taken as a positive result (Harley, 2005).

3.8.6 Catalase test

The catalase test is primarily used to distinguish among Gram-positive cocci. Members of the genus *Staphylococcus aureus* are catalase-positive, while the genera of the *Streptococcus* and *Enterococcus* are catalase-negative (MacFaddin, 1985). A 3% of H_2O_2 was dropped on the slide and small amount of the colony a bacterium was transferred to cleaned surface of slide. A rapid oxygen evolution of oxygen was taken as positive result while absence of bubbles or scattered bubbles was taken as negative result (Table 9).

3.8.7 Carbohydrate Utilization Medium

The medium was prepared according to the manufacturer's instruction and 1% of glucose, mannitol, sucrose and fructose, were added separately to test the extent of organism's metabolic flexibility (MacFaddin, 2000). From the stock of the slant, a tiny amount of growth was inoculated and mixed gently, then incubated for 24-72 hours. Then, the color changes to yellow, and gas bubbles in fermentation were taken positive result of the sugar fermentation and gas production respectively (Table 9).

3.9 Survey Data Collection

Semi-structured questionnaires were used to collect information from dairy products consumers (Appendix 13). The questionnaires were used to collect information on demographic and socio-economic indicators; availability of the dairy products around the home area; frequency of dairy products consumption, and purchasing power. Additionally, the consumers were asked the distance to purchase dairy products, storage methods and handling practices, and potential knowledge on safety and nutritional aspects.

3.10 Statistical analysis

The raw data was inserted into Microsoft Excel and microbial raw data was then transformed to $\log_{10}$ before statistical analysis. Quantitative data were summarized using SPSS software (Statistical Package for Social Science, Version 22) through descriptive statistics like frequency, percentages, ranges, mean, minimum and maximum value. Analysis of variance was done to test the significant difference among the means across the value chain. The mean separations were done by Least Significance Difference (LSD) at ($p \leq 0.05$). For the survey data analysis, the questionnaires were first coded and descriptive statistics were done for each of the categorical questions. The significance difference (non-parametric test) between the responses of each question was done with chi-square through cross-tabulation analysis.

4. Results and Discussion

4.1 Milk Quality

Around one-third (32%) of the milk samples collected across the value chain were positive for alcohol tests, while only 14% of the samples test positive for the clot-on-boiling test (Table 2). The current result of the study showed the majority of raw milk samples collected from producers were negative for alcohol test unlike samples collected from milk collection centers and union gates (Table 2). Among milk samples collected from milk collection and union, nearly 40 and 25% of the samples were positive for alcohol and clot on boiling respectively (Table 2). All the pasteurized milk samples collected from retailers were negative for clot-on-boiling test (Table 2). The raw milk samples collected from the bulk had a lower percentage of negative results either for alcohol test or clot-on boiling test compared to samples collected from individual farmers and retailers (Table 2).

Table 2: Milk quality tests along the milk value chain of the study areas

Milk Value chain		Alcohol test		Clot-on-Boling test	
	Number of the samples (N)	Positive Samples (n)	Percentile (%)	Positive Samples (n)	Percentile (%)
Milk producers	10	2	20	1	10
Milk collectors	10	4	40	3	30
Milk Union	10	4	40	2	20
Raw milk retailers	10	3	30	1	10
Pasteurized milk Retailers	10	3	30	0	0
Overall mean	50	16	32	7	14

The current finding has similarities with a report of Wasiksiri *et al*. (2010) who pointed that among samples tested for milk quality, more samples had positive results for alcohol test compared to the clot-on boiling test. The present finding has also closeness with the report of Haile (2015) who indicated that out of the total milk samples collected from West Shoa Zone, 32.2 and 18.8% were positive with alcohol and clot-on boiling tests respectively. Although, Yilma (2010) reported a similar result (14%) for the positive clot-on boiling test of the milk collected in the central high land, the percentages of positive samples (21%) with alcohol test was lower than the current study result and this might be the difference of acidity content in milk samples.

However, the current report had lower results than the report of Asmnew (2010) who indicated the percentages of positive samples collected from smallholders were 51% and 23% with alcohol and clot-on boiling tests respectively. The difference of the finding may indicate the variation of milk handling practices that affect the milk quality status among the milk actors. Milk quality with positive alcohol or clot on boiling test will not withstand processing conditions like pasteurization or ultra-heat treatment of the milk. Although the exact percentages of the acidity developed might vary for both of the tests, Chaudhry *et al.* (2015) have discussed that samples that were positive on clot-on-boiling and alcohol test might contain acids or acid-producing bacteria and unstable milk protein, respectively. Furthermore, Machado *et al*. (2017) have stated that milk with low stability to ethanol test is considered unsuitable for industrial procedures involving heating and should not be transported to the industry.

Milk acidity and pH had a significant difference across the value chain ($p \leq 0.05$). The overall mean percentages of the milk acidity and pH of the samples were 0.32 and 6.14 respectively (Table 3). However, the cottage cheese contained a higher acidity value (1.32%) and lower pH value (4.34) compared to the milk samples (Table 3 and 4). Regu *et al*. (2015) have reported a similar result with the current study that the pH of the traditional cottage cheese was 4.13%.

The mean percentage of the milk acidity for samples collected from producers, collectors, union, retailers, and pasteurized milk were 0.27, 0.46, 0.42, 0.24, and 0.24, respectively, which ranged from 0.24 to 0.46 (Table 3). The highest mean percentage of the acidity (0.46%) was observed for the samples collected from the bulk tank (collection sites), which was higher by 50% compared to raw and pasteurized milk samples from retailers. The highest mean pH values were observed for samples collected from farmers while the lowest mean value was from pasteurized milk at collection points (Table 3).

The highest pH value indicates the freshness of milk contrast to low pH that is acidified by microorganisms. The milk samples which are taken from the bulk containers had a high value of titratable acidity and low pH value (Table 3). The milk acidity can also determine the suitability of the milk for further processing (heat treatment) and the shelf life of products after processing and it is positively related to milk coagulation conditions. Raw milk can have different processing conditions and the shelf life based on its level of acidity. The natural acidity of the milk may vary depending on species, breed, stage of lactation of cows, the physiological condition of the udder, and storage time and temperature; real acidity reduces the pH value by the degradation of lactose through bacterial activity (Krishnaiah, 2005).

4.2 Proximate Composition

The mean percentages of chemical compositions (fat, protein, lactose, total solid, SNF, and ash) had a significant difference ($p \leq 0.05$) across the dairy value chain. The fat percentages of the milk samples across the value chain ranged between 2.775 and 3.975 and the overall mean value was 3.63 (Table 3).The mean percentage SNF of the milk samples was 8.20, while it was 3.01 for the protein value. The overall means of total solid and lactose were higher by 26 and 40% compared to the value of milk SNF and protein respectively. The mean percentage of ash value of the milk ranged from 0.559 to 0.7500, with the overall mean percentage of 0.66 (Table 3). The mean percentage of the fat, total solids and SNF of cottage cheese were 0.66, 23.32, and 22.59 respectively (Table 4).

In all, the highest percentages of milk compositions were found at the production stage while the lowest mean percentages were observed in pasteurized milk at retailers. Remarkably, the mean percentage of the raw and pasteurized milk at retailers' sites was statistically different and it was considerably reduced by 40% at pasteurized milk (Table 3). The variation of the milk compositions in this study might be emanated from three major factors.

1) The first cluster of factors could include feeding, breed types, age of the animal, lactation interval, and practices after milking. O'Connor (1994) have suggested as cows get older, the fat content of the milk decreases by 0.02 percentage units per lactation, while the fall in SNF is about 0.04 percentages. Bille *et al*. (2009) have also indicated the physicochemical properties were varied between milk samples collected during both morning and afternoon milking in Namibia.

Although this might not be suggested for milk quality across the value chain, it might intensely reflect the reasons for the cases of current milk compositions which are below the Ethiopian standard (ES: 3460:2008). The current finding had a similarity with Workiye (2012) who also reported that fat content of raw milk was 3.74 in Debre Libanose, while Enb & Donia (2009) have reported raw milk compositions like protein and ash, total solid and lactose were 3.20, 0.65, 12.10 and 5.0 respectively in Egypt. Meanwhile, Mohammed *et al*. (2013) reported the raw milk composition of protein, fat, lactose, and acidity as 3.37, 3.73, 4.51, and 0.37 respectively, in Sudan. A study by Alganesh (2016) showed that raw milk composition of SNF, ash, lactose, and fat as 8.56, 0.61, 5.08, and 3.76, respectively in the central highland part of Ethiopia.

The second factor might be related to the high demand and seasonality of milk supply. Besides, dishonest producers and traders could also deliberately adulterate milk and its products to increase the volume which has an impact on the physicochemical properties of milk (El-loly *et al.,* 2013). Adulteration with water is commonly practiced which not only significantly reduces the nutritional value and physical properties of milk (Swai and Schoonman, 2011), but also affect its microbial properties.

The present study result is in agreement with the report of Warsama *et al.* (2017) who found that 20% higher protein value of raw milk from producers compared to the collection and selling points. Mboya & Kurwijila (2017) have also reported that the variation of SNF and fat percentages was observed between the samples collected from different value chains. Haile (2015) also indicated the trend of milk fat, protein, and SNF decreasing from individual farmers to bulk of the milk cooperatives, while Tesfay *et al.* (2015) have reported the total solid of raw milk collected from producers was higher. Estifanos *et al.* (2015) have also indicated the variation of milk chemical composition along the value chain, while Genzebu *et al.* (2016) reported the variation of SNF of the raw milk along the value chain. Meanwhile, Dehinnet *et al*. (2013) have indicated the variation of SNF within raw milk collected across the milk value chain. The variation of milk composition along the value chain may indicate that practices done by value chain actors have the impact on chemical composition. Such practices as adulteration may reduce the nutritional quality of the dairy products and adulterant may also contribute the reduction of microbial safety and quality of the milk and milk products. In some cases, the lactose composition can also be affected by storage condition and by presence of bacteria in milk (O'Mahonny 1998).

Table 3: **Physicochemical properties of the milk across the dairy value chain**

Milk Value chain	Physicochemical properties of the milk							
	Acidity(%)	pH	Fat (%)	TS (%)	Ash (%)	Protein (%)	Lactose(%)	SNF (%)
Milk producers	0.27±0.03[b]	6.49±0.05[a]	3.98±0.16[a]	13.30±0.13[a]	0.75±0.16[a]	3.49±0.53[a]	5.97±0.01[a]	9.47±0.15[a]
Milk collection	0.46±0.02[a]	5.88±0.15[b]	3.83±0.10[a]	11.03±0.11[b]	0.71±0.19[a]	3.19±0.09[b]	5.17±0.24[b]	8.36±0.22[b]
Milk union	0.42±0.02[a]	5.89±0.14[b]	3.40±0.11[b]	11.37±0.15[b]	0.64±0.01[b]	3.06±0.07[b]	4.91±0.16[b]	7.97±0.19[b]
Raw milk retailer	0.24±0.02[b]	6.40±0.09[b]	3.35±0.08[b]	11.88±0.19[c]	0.61±0.01[b]	3.10±0.07[b]	5.12±0.21[b]	8.53±0.15[b]
Pasteurized milk retailer	0.24±0.03[b]	6.02±0.21[b]	2.78±0.06[c]	9.39±0.26[d]	0.55±0.01[c]	2.00±0.07[d]	4.06±0.18[c]	6.62±0.24[c]
Overall mean	0.32±0.015	6.14±0.06	3.5±0.06	11.48±0.14	0.66±0.01	3.01±0.05	5.10±0.10	8.20±0.12

All values are mean ± SE in duplicates; values in the same row with different superscripts are significantly different ($p \leq 0.05$)

The mean percentage of raw and pasteurized milk protein at point of the retailer was significantly different and considerably reduced by 35% in pasteurized milk. Such difference could be attributed to the loss of some chemical components during heat treatments or milk pasteurization. Sakkas *et al.* (2014) have suggested that milk heating can affect its quality and properties through degradation of lactose, denaturation of whey proteins, destruction of vitamins and enzymes, hydrolysis of protein and lipids, and disturbance of calcium/phosphorus equilibrium. The mean percentages of total solid, fat, protein, SNF, and ash of raw milk samples were higher compared to processed (pasteurized) milk samples. Tesfay *et al.* (2015) have revealed that the mean percentages of total solid of raw milk collected from producers were higher related to pasteurized milk from retailers which were similar to the current finding.

The loss of the fat content might be related with fat-soluble vitamins like vitamins A, E and K. Hence, the current finding suggests the fortification of vitamins at the dairy processing industry which is significantly important as they play a vital role in human nutrition and health. The result also indicated insignificant variation ($p \geq 0.05$) of chemical compositions in cottage cheese, despite home-made or factory-made cheese collected from farmers' markets and retailers sites (Table 4). The overall mean value of total solids, ash, and SNF of cottage cheese collected across the value chain was 23.26, 0.66, and 22.59, respectively (Table 4). Cottage cheese collected from retailers found in Addis Ababa contained the highest percentage of total solids and SNF while the lowest value was found in samples collected from Chancho farm market (Table 4).

Table 4: Physicochemical properties of cottage cheese across the value chain

Cottage cheese parameters (%)	Locations			
	Wolmera farm market	Chancho farm market	Addis Ababa retailer	Overall mean ±SE
Acidity	1.46±0.34[a]	1.03±0.19[b]	1.62±0.52[a]	1.37±0.44
pH	4.19±0.39[b]	4.38±0.34[b]	3.83±0.15[c]	4.13±0.38
Total Solid	22.63±1.14[b]	21.60±1.60[b]	25.49±0.69[a]	23.26±2.02
Fat	0.73±0.36[a]	0.70±0.16[a]	0.56±0.10[a]	0.66±0.24
SNF	21.90±1.09[b]	20.95±1.46[b]	24.93±0.66[a]	22.59±2.03
Ash	0.87±0.11[a]	0.89±0.12[a]	0.92±0.14[a]	0.89±0.12

All values are mean ± SE in duplicates; values in the same row with different superscripts are significantly different ($p \leq 0.05$)

The present result of the study had a similarity with the work of Eshitu & Asresie (2019) who reported that the physicochemical properties (total solid) of *ayib* were collected at eastern Gojjam in Ethiopia. However, the authors reported higher results of fat and ash percentages of cottage cheese as compared to the current result of the study. The physicochemical properties of cottage cheese can be affected by several factors like the initial milk composition, the time duration of churning, and production practices.

4.3 Milk and cottage cheese mineral content

Mineral contents of dairy products were varied across the value chain and the percentage of phosphorus, calcium, zinc, and iron values were significantly different ($p \leq 0.05$) across the value chain (Table 5). The raw milk contained a higher value of phosphorus, calcium, zinc, and iron values compared to pasteurized milk. Raw milk collected from milk producers contained higher mineral compositions compared to samples collected from collectors, unions, and retailers. Milk and cottage cheese predominantly contained phosphorus and calcium, but less percentage of zinc, and iron (Table 5).

Table 5: **Mineral composition milk and cottage cheese across the dairy value chain (mg/100g)**

	Minerals			
Dairy value chain	P	Ca	Zn	Fe
Milk producers	0.96 ± 0.01^{b}	1.11 ± 0.02^{a}	0.0070 ± 0.0007^{b}	0.0029 ± 0.0005^{a}
Milk collectors	0.81 ± 0.01^{c}	0.90 ± 0.02^{b}	0.0045 ± 0.0003^{b}	0.0018 ± 0.0001^{b}
Milk Unions	0.74 ± 0.03^{c}	0.85 ± 0.04^{b}	0.0053 ± 0.0014^{b}	0.0012 ± 0.0003^{c}
Raw milk retailers	0.94 ± 0.02^{b}	1.06 ± 0.03^{a}	0.0055 ± 0.0004^{b}	0.0018 ± 0.0003^{b}
Pasteurized milk retailers	0.72 ± 0.02^{c}	0.88 ± 0.01^{b}	0.0024 ± 0.0003^{b}	0.0017 ± 0.0003^{c}
Farm markets cottage cheese	1.12 ± 0.02^{a}	0.80 ± 0.04^{c}	0.0231 ± 0.0043^{a}	0.0007 ± 0.0001^{d}
Retailer markets cottage cheese	1.11 ± 0.03^{a}	0.74 ± 0.03^{c}	0.0176 ± 0.0042^{a}	0.0007 ± 0.0001^{d}
Overall mean (Mean ±SE)	0.92±0.02	0.90±0.09	0.0096±0.0012	0.0016±0.0001

Values are Mean ± SE in duplicates, Mean ± SE with different superscripts in the same column are significantly different ($p \leq 0.05$)

The current study more or less had a similarity with the finding of Soliman (2005) who reported the minerals composition of cow milk 119 mg (Calcium), 95 mg (Phosphorus), 0.07 mg (iron), and 0.38 mg (Zinc) in 100 g sample. The findings of the authors and the current study agreed with the trend minerals composition between milk and cottage cheese as suggestively varied and the calcium content was decreased pronouncedly compared to milk. Although the similarity of results was observed between the current finding and Vahčić *et al*. (2010) for milk calcium and phosphorus, the concentration of iron and zinc of the current result was lower. Elfdial (2016) have reported the phosphorus and calcium content of raw milk as 85 and 117 mg within 100 g, however, they reported that cottage cheese contained higher phosphorus and lower calcium compared to raw milk.

4.4 Microbial quality of milk and cottage cheese

The current study indicated that the samples tested positive across all dairy products value chain (Table 6). The highest frequency of positive samples of *Salmonella* and *Listeria* spp. was found in raw milk samples collected from the union gate. The raw milk samples collected from the milk union gate had a high chance of contamination by these pathogenic organisms compared to the samples collected from the producers and retailers. The result of the study indicated among 10 raw milk samples collected from the collection sites, the percentages of milk contamination by *Salmonella* and *Listeria* spp. were 30 and 50% respectively. The prevalence of *Salmonella* and *Listeria* spp. of the cottage cheese collected from Addis Ababa retailers was 0 and 10%, respectively, which was minimally contaminated. The prevalence of *Salmonella* spp. was found in cottage cheese samples collected from farm markets or producers was 20% (Table 6).

The current result of the study was comparable with the report of Tadesse & Dabassa (2012) who found 35.5% in raw milk collected from farmers in Jimma Zone, and the report of Desta *et al.* (2011) who indicated that the prevalence of the *Listeria* spp. 43.1% at Asella and Debrezeit, but Ejo *et al.* (2016) reported the absence of *Salmonella* spp. in cottage cheese in Gondar City. Meanwhile, Omar *et al.* (2018) have reported 16% of *Salmonella* spp. prevalence from fresh cheese collected from street vendors in Egypt. Additionally, in Addis Ababa, a 1.6% prevalence of the *Listeria* spp. was reported (Molla *et al.*,2004).

The prevalence of *Listeria* spp. in milk and cottage cheese was compared by Muhammed *et al.* (2013) who reported 14 and 2% *Listeria* spp. in milk and cottage cheese respectively in Jimma but also, Garedew *et al.* (2015) indicated 14 and 12.5% of *Listeria* spp. prevalence in raw and cottage cheese collected in Gondar City.

Table 6: Prevalence of *Salmonella* and *Listeria* spp. across the dairy products value chain

Dairy products Value chain	*Salmonella* spp.			*Listeria* spp.	
	Number of the samples (N)	Positive samples (N)	Percentile (%)	Positive samples (N)	Percentile (%)
Milk producers	10	4	40	4	40
Milk collectors	10	3	30	5	50
Milk Unions	10	5	50	4	40
Raw milk (retailers)	10	3	30	2	20
Pasteurized milk (retailers)	10	0	0	2	20
Cottage cheese (open markets)	10	2	20	3	30
Cottage cheese (retailers)	5	0	0	1	10
Overall mean	65.0	17	24.28	21	30.0

The prevalence of *Salmonella* and *Listeria* spp. was higher in raw milk samples than pasteurized milk samples. Remarkably, the current finding showed a 20% prevalence of *Listeria* spp. was found in pasteurized milk collected from the retailers. Seyoum *et al.* (2015) reported the prevalence of *Listeria* spp. three times greater than the current finding. However, several scholars (Garedew *et al.*, 2015; Fisseha, 2017) reported the absence of *Listeria* spp. in pasteurized milk.

The current finding disagreed with the reports of (Dailey, 2011; Abunna *et al.*, 2018; Banti, 2018) who reported the absence of the *Salmonella* spp. prevalence collected from individual farmers and the bulk of the raw milk. However, the current study results indicated the prevalence of *Salmonella* spp. collected from milk producers lower compared with Tesfay *et al.* (2013) who reported 44.4 % prevalence of *Salmonella* spp. found in producers.

The current finding indicated *Listeria* spp. contamination of cottage cheese was lower by one-third compared to Fisseha (2017) who reported 86% of cottage cheese samples collected from Bishoftu and Dekum tows in the Oromia region. Seyoum *et al*. (2015) have also reported around 52% of cottage cheese samples were contaminated by *Listeria* spp. which was by far higher than the current finding of the study.

The prevalence of both *Salmonella* and *Listeria* spp. can be varied which may reflect the individual practices, water quality used to clean milk equipment; personal hygiene of the milkers, hygienic status of milk equipment, storage temperatures of milk at the gate of the milk collections and unions, and milking environments. Mulu & Pal (2016) have revealed that animal products collected from high-risk factors were related to the high prevalence of *Listeria* spp. and *Listeria monocytogenes*. Less contamination of cottage cheese compared to raw milk might be due to cottage cheese processing temperatures. But also, exposure of raw milk to lactic acid fermentation before local cottage cheese processing may reduce initial raw milk contamination.

Although, pasteurization is believed to destroy the vegetative cells of pathogenic organisms, several reports show (McLauchlin *et al*., 2004; Todda & Notrmansb, 2010) that the *Listeria* spp. can be found in raw and processed food (milk and dairy products). But this happens when the overall hygienic condition is poor at milk processing, machines, aprons, ripening premises, cold stores, open space, packaging materials, etc. The prevalence of *Listeria* and *Salmonella* spp. may relate to fault-pasteurization or post- contamination during handling practice of shops, transportation systems, and storage temperatures. Additionally, the current finding suggests the final quality of pasteurized milk can be affected by the overall microbial quality of producers, collectors, retailers, and other value chain actors of the milk.

The microbial load of milk and cottage cheese had a statistical significance difference ($p \leq 0.05$) along the value chain. The overall $\log_{10}$ CFU/ml mean of *Staphylococcus aureus* and total coliform of the raw milk samples were 5.35 and 6.17 $\log_{10}$CFU/ml respectively (Table 7).

The mean *Staphylococcus aureus* count of pasteurized milk was ranged from 1.68 to 3.70 $\log_{10}$ CFU/ml and the overall mean value of 2.68 $\log_{10}$CFU/ml. It was by half lower than raw milk contamination. The mean $\log_{10}$ CFU/ml of the *Staphylococcus aureus* and total coliform counts of the raw milk collected at milk union gates was 6.52 and 7.07, respectively, which was the

highest milk contamination point (Table 7). But, the result showed that it was reduced by nearly 43% in pasteurized milk collected from retailers. Remarkably, the meanlog_{10}CFU/ml of the total coliform count and *Staphylococcus aureus* of the raw milk collected from collection sites and union were insignificant ($p \geq 0.05$) and the same scenario was also observed between raw retailers and producers. The least raw milk contamination point was observed at the milk production stage (Table 7).

Table 7: **Microbial quality (mean log_{10}CFU/ml) of milk across the milk value chain**

Value chain	Staphylococcus aureus	Minimum	Maximum	Total coliform	Minimum	Maximum
Milk producers	4.02 ± 0.61^{b}	ND	5.85	5.34 ± 0.31^{c}	4.31	6.81
Milk Collectors	5.93±0.75a	5.59	6.29	6.33 ± 0.14^{b}	5.80	7.06
Milk Unions	6.52 ± 0.22^{a}	5.48	7.30	7.07 ± 0.31^{a}	5.35	8.16
Raw milk (retailers)	4.94 ± 0.28^{b}	3.30	5.89	5.94 ± 0.24^{c}	4.88	6.84
Pasteurized milk (retailers)	2.62 ± 0.32^{c}	1.68	3.70	3.32 ± 0.27^{d}	1.57	4.19
Mean	4.89±0.24	-	-	5.70±0.20	-	-

Values are Mean ± SE in duplicates, Mean ± SE with different superscripts in the same column are significantly different ($p \leq 0.05$)

The microbial quality of cottage cheese collected from different value chain had statistically significant variations ($p \leq 0.05$). The microbial load of the cottage cheese (total coliform and *Staphylococcus aureus*) collected from Chancho farm market was the highest compared to Wolmera farm market and Addis Ababa cottage cheese retailers (Table 8). The mean log_{10}CFU/g of total coliform of cottage cheese samples collected from Addis Ababa retailers was by far the lowest (1.63 log_{10} CFU/g). The mean range of *Staphylococcus aureus* of the cottage cheese collected from three locations of the value chain was ranged from 3. 44 to 6.82log_{10} CFU/g and the overall mean was 4.98log_{10} CFU/g (Table 8).

Table 8: **Microbial quality (Mean log_{10}CFU/g) of cottage cheese across the value chain**

Cotta cheese Value chain	Total coliform	Minimum	Maximum	*Staphylococcus aureus*	Minimum	Maximum
Wolmera farm markets	3.40±0.92a	ND	4.95	4.42±0.33b	3.44	5.18
Chancho farm markets	4.07±0.26a	3.13	4.60	6.50±0.12a	6.06	6.82
Addis Ababa retailers	1.63±0.10b	1.26	1.86	4.98±0.31b	3.80	4.19
Overall mean	3.03±0.40	-	-	4.98±0.31	-	-

Values are Mean ± SE in duplicates, Mean ± SE with different superscripts in the same column are significantly different ($p \leq 0.05$)

Massawe *et al.* (2019) have reported similar to the current findings since the load microorganisms of increased from producers to collection points. The current mean range of the total coliform count result (3.32 to7.07 log_{10}CFU/ml) was comparable with several findings (Bareda *et al.*,2012; Tesfay, 2015; Estifanos *et al.*, 2015; Warsama, 2018; Gwandu *et al.*, 2018; Nalwaya *et al.*, 2018) who reported within the range of the current result of the study. The present finding was supported by the work of Regasa *et al.* (2019) who reported 15.3% of 183 milk samples collected in the central highland of Ethiopia (Mukaturi and Sululta), where contaminated by *staphylococcus aureus*. But also, Shunda *et al.* (2013) reported that nearly one-fourth of the raw samples collected from the Mekele dairy value chain was contaminated by *Staphylococcus aureus*.

The microbial load of cottage cheese of the current result was lower compared to the report of Zelalem *et al.* (2011) who reported 4.4log_{10}CFU/g. The current finding had a similarity regarding the *Staphylococcus aureus* contamination with the report of Haddad & Yamani (2017) who found a 6.5log_{10}CFU/g microbial load of traditional soft white cheese collected from the different value chain of Jordan.

Staphylococcus spp. may be found on the skin and mucous membranes of healthy warm-blooded animals, as well as in soil, air, and water and it is a pathogenic organism that is commonly found in food items with poor hygienic practices and equipment, human contacts during marketing and processing and the environment where animal housed and milked dairy cows are major sources of possible contamination (Asperger & Zangerl, 2001; Bonfon *et al.*, 2003; André *et al.*, 2008; Dufour *et al.*, 2012).

Table 9: Biochemical tests of bacteria isolated from milk and cottage cheese

Isolates	Gram	Catalase	Gas	H_2S	Citrate	Motility	Glucose	Sucrose	Mannitol	Spore	Fructose	TSI
Salmonella spp.	-	-	+	+	+	+	+	-	+	-	+	y/y
	-	-	+	+	+	+	-	-	+	-	+	y/y
	-	-	+	+	+	+	+	-	+	-	+	y/y
	-	-	+	+	+	+	+	-	+	-	+	y/y
	-	-	+	+	+	+	+	-	+	-	+	y/y
Listeria spp.	+	+	-	-	-	+	+	+	-	-	+	y/y
	+	+	-	-	-	+	+	+	-	-	+	y/y
	+	+	-	-	-	-	+	+	+	-	+	y/y
	+	+	-	-	+	+	+	-	-	-	+	y/y
S.aureus	+	+	-	-	+	-	-	+	+	-	-	y/y
	+	+	-	-	+	-	+	+	+	-	+	y/y
	+	+	-	-	+	-	+	+	+	-	+	y/y

Positive results (+), - Negative results (-), y/y glucose, lactose plus sucrose fermented

4.6 Dairy Food Environments

4.6.1 Population Socio-demographic Profile

Among 120 respondents, the percentage of male and female involved in the survey were 43.3 and 57.7 respectively (Table 10). Regarding the marital status of the respondents, many of them was 73.3% married, while 12.5 and 14.2% were single and widow, respectively. The highest proportion of educational background of the respondents was high school (41.7%) while the least of the percentages of respondents were basic education and post-graduate status (Table 10).

The percentage of respondents (33.3%) who attended elementary school was one-third of the total respondents and those who attended diploma or above were only 21.7%. Out of the ages of the respondents, 56% were found between 31-45 ages while surpasses one-third of them found between 18-30 ages (Table 10). The rest of the respondents (12%) were found above 45 years old. The highest proportion of the age of the respondents was ranged 31-45 years which accounts for about 56.7% while nearly one-third of total respondents were found between 18-30 years. However, the least percentages (10.8) of respondents are found at 45 years and above (Table 10).

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Table 10: Sex, marital status, ages, and educational status of the respondents

Variables	Total Number of respondents (N)	Frequency (N)	Percentages (N %)
Sex of the respondents	120		
Male		52 (120)	43.3
Female		68 (120)	57.7
Marital Status	120		
Married		88 (120)	73.3
Single		15 (120)	12.5
Widow		17 (120)	14.2
Age	120		
18-30		39(120)	32.8
31-45		67(120)	56.3
46 and above		13(120)	10.9
Educational Background	120		
Read and Write		4 (120)	3.3
Elementary School (1-8)		40 (120)	33.3
High School (9-12)		50 (120)	41.7
Certificate/Diploma		18 (120)	15.0
Degree and Above		8 (120)	6.7

4.6.2 Consumption of habit of the Dairy products

The result indicated that there was a significant difference ($p \leq 0.05$) between the responses of the respondents on the frequency of dairy product consumption. The survey result indicated that nearly 56 % of the respondents had dairy products in their homes, while 20 % of them responded as dairy products available bi-weekly (Table 11). However, the least percentages of respondents answered as weekly and occasional were 14.2 and 11.7% respectively. Most of the respondents were consumed dairy products during the non-fasting season and the least of respondents consumed occasionally only during the non-fasting season (Table 11). Contrary, 38.3% of the respondents responded as they consumed dairy products at all times regardless of the fasting season (Table 11).

Almost third-fourth of the respondents responded as they purchased or had the dairy product store at their home within a week and most of them practicing daily (Table 11). The current finding had a similarity with the survey result reported on the average per capita milk consumption of Addis Ababa city was very high (Tamirat, 2019).

The result of the current study indicated a significant response among the respondents regarding the importance of milk and milk products intakes critically for children over the other age group of the population (Table 11). A 40% of them had an opinion from 6 months to 10 years children should get the milk, however, 29.2% of respondents believed from 6 months to 2 years (Table 11). Contrary, around 77.5% of the respondents did not include any dairy products in their lunch while they go to school. But, nearly one-quarter of the respondents practiced to include dairy products in their school lunch (Table 11).

The potential knowledge of knowing the importance of dairy products has positive implications for nutrition improvement strategies. Watson *et al*. (2006) have explained better nutrition status for those consumed cow milk-based on the survey result conducted at South Wello, the northern part of Ethiopia. The result of the survey also indicated statistically significant variations of dairy products and pasteurized milk consumption by children. Nearly, above 50% of the respondents agreed as boiled pasteurized milk was widely consumed by children (Table 11).

However, the result indicated the percentages of the dairy products like cheese, ice cream, yogurt, and others (20%) consumed were by far less compared to fresh boiled raw milk (45%) (Table 11). The result reveals that dominantly families decide purchasing dairy products for their children and few of those (0.8%) responded children buy dairy products by themselves very insignificant. But also, the result indicates that relatives and neighbors (5.8%) had involvement in dairy products purchasing for food choices of the children (Table 11).

The collective results of the current study indicate dairy processing might have a positive implication on dairy sector development and the food system of the country. The dairy food system encourages activities of milk production, processing and packaging, retailing, safety and quality, and dairy product availability, but also a positive consequence on nutrition and health outcomes. The result revealed that dairy products intake and hypothesized for nutrition, and the milk products like cheese, yogurt, cream, etc. were consumed less likely by pre-school children, although, it was significantly consumed by adolescence.

Contrast to current finding Herrador *et al.* (2015) reported that low animal source food consumption of pre-school children in Limbo Kemkem and Fogera Districts of Ethiopia and they also indicated by far the variation of animal source foods between urban and rural within the locations.

Table 11: Dairy products consumption habit of the households and pre-school children

Categorical variables	Responses	Frequency	Percentage	Significant test ($\chi 2$)
Frequency of dairy product consumption habit	Daily	67/120	55.8	**
	Bi-weekly	24/120	20	
	weekly	17/120	14.2	
	Occasional	14/120	11.7	
Frequency of dairy products consumption habit and seasons of the year	Always	46/120	38.3	**
	Only in non- fasting season	70/120	58.3	
	Sometimes during in non-fasting season	2/120	16.7	
Dairy products for pre-school children and awareness of respondents	Yes	113/120	94.2	**
	No	7/120	5.8	
Perceptions and the ages of children	6 months- 2 year	35/120	29.2	**
	6 months- 7 year	22/120	18.3	
	6 months – 10 Year	15/120	12.5	
Dairy products and lunch of pre-school children	Yes	27/120	22.5	**
	No	93/120	77.5	
Dairy products and consumption by pre-school children	Raw milk boiled	34/120	28.3	**
	Pasteurized milk (Boiled)	62/120	51.7	
	Cheese and yoghurt	24/120	20	
Dairy products and consumption by Adolescence	pasteurized milk	48/120	40	**
	Raw fresh milk boiled	18/120	15	
	Cheese, yoghurt, ice cream, and other dairy products	54/120	45	
Choice of pre-school children and dairy products	Families	112/20	93.3	**
	By themselves	1/120	0.8	
	Neighbor or relatives	7/120	5.8	

*Responses were evaluated in terms of frequency and ** indicates a significant difference between the responses with $\chi 2$ ($p \leq 0.05$)*

Dissimilarities may be emanated from the status of study location which indirectly related to the educational background of the family's children. Whenever the milk is processed into different products, there is a possibility of nutrient variation across the dairy products. According to Hop (2003), whole milk was better compared to milk products by both macro and micronutrients, fermented milk products were by far better than whole milk by its probiotic beneficiary bacteria. Higher intake of milk and milk products source foods were associated with better growth, micronutrient status, cognitive performance, and motor development in developing countries, although authors referred to more pronounced cognitive function in children consuming the meat compared to the milk (Allen & Dror, 2011).

But, the theory of children ages and malnutrition was indicated by different International Organizations in 2011 globally as 165 and 52 million of the children were found stunted and wasted respectively and high prevalence was found in Africa and the Asian continent (WHO, 2012). Although, the current survey result indicated only 69.2% of the respondents were significant with FAO (2013) recommendation which children between the ages of 6-18 months are a critical age of children's lives and they should get ASF and other complementary diets Because of their fast-growing rate ages.

This may be due to several factors derived from an educational background, income, nutrition knowledge, and culture, or religion of the child's families. A recent finding by Potts *et al.* (2019) revealed that young children in Ethiopian Orthodox households were less likely to consume milk and milk products including other animal source food than Muslim religious households.

But also, so far research findings indicated that the fasting implication in which Orthodox Christians of the mother’s households did not want to prepare food from animal source foods while adults in households were fasting due to fear of contamination (Kumera *et al.*, 2018). So, to improve dairy products and other animal source foods consumption of the children, it will be better if religious leaders and families get the nutritional knowledge of dairy products.

4.6.3 Dairy products safety

The majority of the respondents (89.2%) had awareness of checking the shelf life of the dairy products while they bought from supermarkets or shops, irrespective; a few of the respondents had no awareness (Table 12). However, respondents were used different sensory evaluation techniques like smell and taste to evaluate whether the dairy product fit for consumption or not. There was a highly significant difference across respondents based on dairy product storage methods (Table 12).

To ensure food safety different actors play a key role and among them, consumer awareness is critical to ensure food safety. Azanaw *et al*. (2019) reported as the majority of the respondents had food safety knowledge, attitude, and practice in general although too few of them are familiar with pathogenic microorganisms that are common in milk and milk products.

Food storage is also another important term in food safety in the food system scenario. The study indicated that nearly 68% of the respondents used refrigerator to preserve milk and milk products and 4.2% of the respondents used the cold water traditional way of dairy products preservation system. But, nearly 29.2% of respondents never use any preservation mechanism rather use the products within an hour after purchase. Approximately equivalent proportions of the respondents (39.2 and 41.7%) allowed dairy product storage time before consumption for an hour or a day. However, a few of the respondents (4.2 %) stored dairy products for more than two days. Regarding dairy safety, about 97.5% of the respondents answered as they had a worry of milk adulteration which might be intended to increase the volume of milk (Table 12). In this study, there was a significant difference among the storage methods of the respondents and most of them used refrigerator which is recommended for food storage and the majority of them allow purchased products only for a day.

Table 12: **Dairy products handling practices and safety**

Variables	Responses	Frequency	Percentage	Significance test ($\chi 2$)
Dairy products purchasing and awareness of shelf-life	Yes	107/120	89.2	**
	No	13/120	10.83	
Traditional methods and Safety of dairy products	Color	0/120	0	**
	Smell	8/120	6.7	
	Taste	5/120	4.2	
Storage methods and consumption	Refrigerator	80/120	66.7	**
	Coldwater	5/120	4.2	
	Upon using	35/120	29.2	
Storage time and consumption	For an hours	47/120	39.2	
	For a day	50/120	41.7	**
	For a days	18/120	15	
	> two days	5/120	4.2	
Milk adulteration trend and perception of the consumers	Yes	117/120	97.5	**
	No	3/120	2.5	

*Responses are evaluated in terms of frequency and ** indicates a significant difference between the responses with $\chi 2$ ($p \leq 0.05$*)

Almost nearly all of the respondents were had similar answers as they worried about milk adulteration in Addis Ababa city (Table 13). Consumer safety awareness plays important role in dairy product safety. The majority of the respondents 87.8% strongly agreed that milk adulteration has harmful effects on health. The current finding is supported by FAO (2013) which stated, although there is no clear evidence between education and knowledge with nutrition, it creates an awareness and positive impact on nutrition and food choices but also good health outcomes.

The result indicated that there was a significant difference between the respondents whether to check or not the nutrition value when dairy products are purchased and most of the respondents had basic understanding of the nutritional value of the dairy products. Among the 120 total respondents, 69.2% had some knowledge of the nutritional value of the dairy product when they purchased 30.8% did not prove the nutritional fact of any products. Although whey is one important dairy product and rich nutrient of mineral and vitamins, almost all of the respondents did not know the value of it (Table 13).

The present finding results indicated respondents used a different source of protein to replace milk and milk products. However, dairy products protein can be seen through whey protein and casein protein. Although whey is one of the nutrient-rich, none of the respondents knew the nutritional value of whey, although it is rich in mineral, vitamins, and overall high biological availability. Respondents were asked food items they can replace in the scarcity of milk and milk products supplies and the result indicates, more than half of the respondents believed that egg protein could replace dairy products while the least percentage of them believed that, soya milk (plant-based protein) next to meat protein. By far a few respondents answered milk powder and this indicates, the consumers may prefer locally other available animal source foods, unlike milk powder which is imported from abroad. Worku (2012) suggested the key to nutrition decreasing was the governmental policy under GTP II focused to reduce malnutrition from 46 to 26 % through dairy product consumption. WHO (2009) suggested that animal source food consumption prevents vitamin A and iron deficiency, and protein-energy malnutrition.

Table 13: Perception of the respondents on nutritional value of the dairy products

Variables	Responses	Frequency	Percentage	Significance test ($\chi 2$)
Knowledge of nutritional value and dairy products	Yes	83/120	69.2	**
	No	27/120	22.5	
Nutritional value of whey and awareness of the consumers	Yes	10/120	8.3	**
	No	110/120	92.2	
Dairy products and other protein foods (Substituting)	Egg	67/120	55.8	
	Meat	15/120	12.5	
	Soya milk	5/120	4.2	
	Milk powder	11/120	9.2	**
	Egg and Meat	13/120	10.8	
	Soya milk	4/120	3.3	

*Responses are evaluated in terms of frequency and ** indicates a significant difference between the responses with $\chi 2$ ($p \leq 0.05$)*

4.6.4 Dairy Product availability and accessibility

There was a significant difference between physical accesses to dairy products of the respondents ($p \leq 0.05$). Around 74.2% of the total respondents can purchase dairy products within a 200 m distance from their home while a few of them nearly 5% cannot get dairy products within a kilometer radius (Table 14). Parallel, 72.5 % of the respondents answered as there were dairy products suppliers that are close to their home living area. The frequency of suppliers was a statistically significant difference (Table 14). FAO (2008) proposed that the physical distance for accessing food is one of the components in the food system. A significant response of the respondents was confirmed of getting the dairy product to be purchased within 200m away from their home areas while very few of them cannot get within that of the distance. Interestingly, the respondents answered the availability of milk and milk products as the most dairy products supplier like milk shops, supermarkets, and dairy products outlets are found in the living area. Dairy products suppliers or marketers have a significant role in the availability of milk and milk products mostly for the urban population.

Table 14: **The accessibility of dairy products and frequency suppliers across the seasons**

Variables	Responses	Frequency	Percentage	Significance Test ($\chi 2$)
Distance to purchase and dairy products	Nearly 200M	89/120	74.2	**
	Nearly 500M	27/120	22.5	
	Nearly 1Km	6/120	5	
	More than 1Km	3/120	2.5	
Cost of dairy products	Cheap	0/120	0	**
	Expensive	96/120	80	
	Fair	24/120	20	
Dairy product accessibility	Yes	113/120	94.2	**
	No	7/120	5.8	
Dairy products and supplier nearby living area	Yes	87/120	72.5	**
	No	13/120	10.8	
Frequency of suppliers and availability of dairy products	Always	85/120	70.8	**
	Only non-fasting Season	7/120	5.8	
	Occasional	7/120	5.8	
Monthly income and the price of the dairy products	No at all	113/120	94.2	**
	Fair	6/120	5	
	Very Fair	1/120	0.8	

*Responses are evaluated in terms of frequency and ** indicates a significant difference between the responses with $\chi 2$ ($p \leq 0.05$)*

The current result supported the report of Ahmed *et al*. (2018) who mentioned the importance of dairy products marketers as two-third of animal source food consumption is acquired from the markets and the situation helped the consumers to get dairy food nearby their home area. The closeness of dairy products suppliers to the home area might also positively impact dairy product consumption and health outcomes. They are several factors affecting consumer behavior, among those, food accessibility to home area, food price and availability, and culture positively affect the consumption habit (Hoang, 2009).

The survey data indicates that the majority of the respondents can get the dairy product from their environment within accessible physical distance although some of them could not get these opportunities. The current result of the survey supported by Zelalem *et al*. (2011) who reported that as a variety of the milk and milk products types like cheese, yogurt, and butter, fresh and processed milk which are local as well as imported products were available in different corners of Addis Ababa City.

There was a significant difference in dairy product accessibility which influences the frequency of dairy product consumption ($p \leq 0.05$). The majority of the suppliers were not time-dependent to provide dairy products. However, nearly 6 % of the respondents agreed as dairy products suppliers only provided in non-fasting seasons. Almost all respondents were thought that dairy product accessibility has a direct relation to dairy product consumption. However, the differences between the responses of respondents regarding the cost of dairy products were most pronounced. None of the respondents responded the cost of dairy products was cheap, although 24 of 120 of them answered as the cost of dairy products is fair. Around 94 % of the respondents answered that the cost of dairy products is significantly unbalanced with their monthly income.

These terminologies (food availability and physical distance access) had positive implications in the food system. Statistically significant ($p \leq 0.05$) responses of the respondents believed that dairy product availability on the markets could affect their dairy products consumption habit. The study suggested that by considering the report of the Bezie (2019) which reported a significant amount of the milk and milk products imported to the country across the years and the domestic production played a vital role in dairy product food availability in the City.

The result also indicated that there were significant responses regarding milk and milk products available across the years despite a few of the milk suppliers were not provided during the fasting season. Despite dairy product availability and physical distance, the survey data revealed that, pronounced the price of the dairy products related to the monthly income of the respondents. This indicates although there is a possibility of dairy products available, it is mismatched with the income. The current finding was supported by Land O' Lakes (2010) which showed that the percentages of milk and milk products consumed by top earners were around 38% while the lowest income consumed only 23%. Incredibly, the finding indicated the ratio of top earners to the lowest-income population was nearly one person from a total of the six people (1:6) in Addis Ababa city of the population.

According to a study conducted on food security determinants assessment among households in Addis Ababa City, around 58.16% of the total households are below the food insecurity line (Gebre, 2012). Zelalem *et al.* (2011) also reported the main determinant for dairy product consumption for the rural and urban population was herd ownership and the monthly income respectively. But, other researchers had a view of increasing the mean consumption of animal source food across the years stimulated the dairy product price to increase in Ethiopia (Bachew *et al.*, 2017).

The current result was related to IFPRI (2008) reported as higher food prices can affect radically across the countries and food intake of the population regardless of food type. FAO (2008) also reported that food prices have serious consequences on purchasing power and especially it affects rural landless, small-scale farmers and urban poor peoples and specifies the situation as food crises. Accordingly, the current result of the study supports the recommendation of the IFPRI (2008) for Ethiopia which was stated as decision-makers should acquire the information and applying methods for understanding which affect food crises in their countries and acting to alleviate the risks and exploit the opportunities brought about such crises.

5. Conclusion

This study demonstrated a moderately raw quality fit for milk-pasteurization, despite too few percentages fit UHT milk processing which produces a long time shelf-life of treated milk.

The chemical composition of raw milk varied among the value chain and most of milk composition of milk are below Standards. The chemical composition of pasteurized milk was lower compared to the raw milk. Milk samples had higher phosphorus and calcium, and lower zinc and iron.

Milk and cottage cheese samples were microbial contaminated and the consumption of raw milk has a health risk for the consumers. In all the study indicated critical calling of food safety and possible interventions to ensure the safety and quality along the dairy value chain. Although dairy products are available and physically accessible, consumption is highly affected by the price and seasons of fasting. Milk production of nearby towns, milk processing plants, supermarkets, and shops played a significant role in the dairy food system of Addis Ababa.

The educational status of the respondents reflected basic knowledge on the importance of dairy products with the concept of nutrition and dairy product handling practices, although less awareness of milk products like whey utilization. The study demonstrated the consumption of dairy products by pre-school and above can be affected by the income of individual households, which negatively affect the nutrition of the children.

6. Recommendations

- Organizing dairy products to formal channels and applying the quality control system at milk collection and processing will provide a better food safety system for the country.

- Dairy product fortification with iron and zinc can combat malnutrition-related to iron or zinc deficiency

- Molecular confirmation through Polymerase Chain Reaction (PCR) can reduce labor and time, and contribute recent science for food safety in placement.

- Loss of essential milk nutrients (vitamins) can happen in pasteurization and further studies need to see the effect of local processors on nutritional value.

- Further studies needed to identify the risk factors or critical control points of local fault milk pasteurization.

- Since, the dairy food environment rely on the dairy food system, increasing milk production, processing and packaging, encouraging investments in Animal Source Food, dairy product diversifications in the shop and supermarkets across the country may balance demand and supply, increase dairy products consumption and positively react with mal-nutrition

7. References

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APPENDICES

Appendix 1: ANOVA table for acidity and pH of the milk

Parameter	Source	Sum of the squares	DF	Mean square	F value	Pr>F
Acidity	Treatment	.862	4	.215	13.564	≤0.001
	Error	1.445	91	.016		
	Total	2.307	95			
pH	Treatment	6.669	4	1.667	4.484	≤0.05
	Error	33.838	91	.372		
	Total	40.508	95			

Appendix 2: ANOVA table for chemical compositions of the milk

Parameter	Source	Sum of the squares	DF	Mean square	F value	Pr>F
Fat	Treatment	15.748	4	3.937	15.825	≤0.001
	Error	22.640	91	0.249		
	Total	38.387	95			
Total solid	Treatment	143.439	4	35.860	61.745	≤0.001
	Error	52.851	91	0.581		
	Total	196.290	95			
Protein	Treatment	21.888	4	5.472	50.291	≤0.001
	Error	9.901	91	0.109		
	Total	31.789	95			
Lactose	Treatment	33.414	4	8.354	11.328	≤0.001
	Error	67.106	91	0.737		
	Total	100.520	95			
SNF	Treatment	75.743	4	18.936	25.824	≤0.001
	Error	66.726	91	0.733		
	Total	142.469	95			
Ash	Treatment	0.426	4	0.106	18.376	≤0.001
	Error	0.527	91	0.006		
	Total	0.953	95			

Appendix 3: ANOVA table for Acidity and pH of cottage cheese *('ayib'*)

Parameter	Source	Sum of the squares	DF	Mean square	F value	Pr>F
Acidity	Treatment	1.854	2	0.927	6.528	≤0.05
	Error	3.834	27	0.142		
	Total	5.688	29			
pH	Treatment	1.547	2	0.774	7.884	≤0.05
	Error	2.649	27	0.098		
	Total	4.196	29			

Appendix 4: ANOVA table for the chemical composition of the cottage cheese *('Ayib'*)

Parameter	Source	Sum of the squares	DF	Mean square	F value	Pr>F
Fat	Treatment	0.165	2	0.082	1.439	≥0.05
	Error	1.545	27	0.057		
	Total	1.710	29			
Total Solid	Treatment	79.588	2	39.794	27.235	≤0.001
	Error	39.451	27	1.461		
	Total	119.039	29			
Ash	Treatment	0.014	2	0.007	0.444	≥0.05
	Error	0.424	27	0.016		
	Total	0.438	29			
SNF	Treatment	86.376	2	43.188	34.399	≤0.001
	Error	33.899	27	1.256		
	Total	120.275	29			

Appendix 5: ANOVA table for the mineral composition of milk and cottage cheese *('ayib'*)

Parameter	Source	Sum of the squares	DF	Mean square	F value	Pr>F
Phosphorus	Treatment	1.523	6	0.254	40.181	≤0.001
	Error	0.385	61	0.006		
	Total	1.908	67			
Calcium	Treatment	1.079	6	0.180	15.971	≤0.001
	Error	0.687	61	0.011		
	Total	1.766	67			
Zinc	Treatment	3562.620	6	593.770	10.030	≤0.001
	Error	3611.130	61	59.199		
	Total	7173.750	67			
Iron	Treatment	34.321	6	5.720	6.504	≤0.001
	Error	53.647	61	0.879		
	Total	87.967	67			

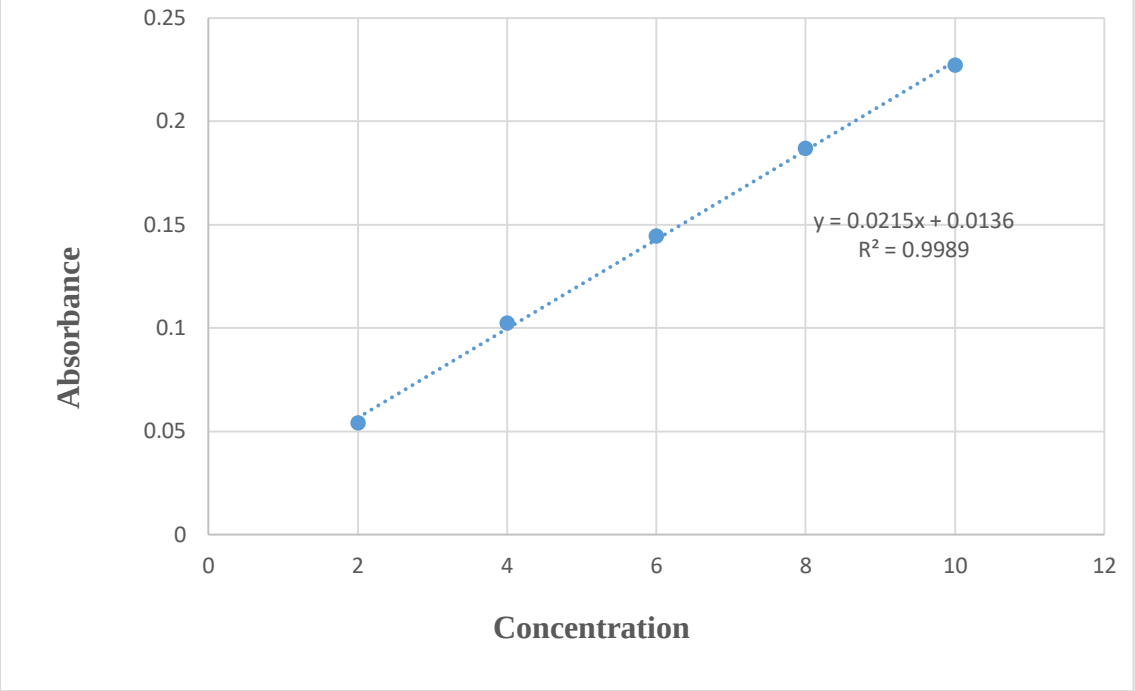
Appendix 6: ANOVA table for total coliform and *Staphylococcus aureus* of milk

Parameter	Source	Sum of the squares	DF	Mean square	F value	Pr>F
S.aureus	Treatment	86.528	4	21.632	17.983	≤0.001
	Error	51.726	43	1.203		
	Total	138.254	47			
Total coliform	Treatment	70.075	4	17.519	25.952	≤0.001
	Error	29.027	43	0.675		
	Total	99.102	47			

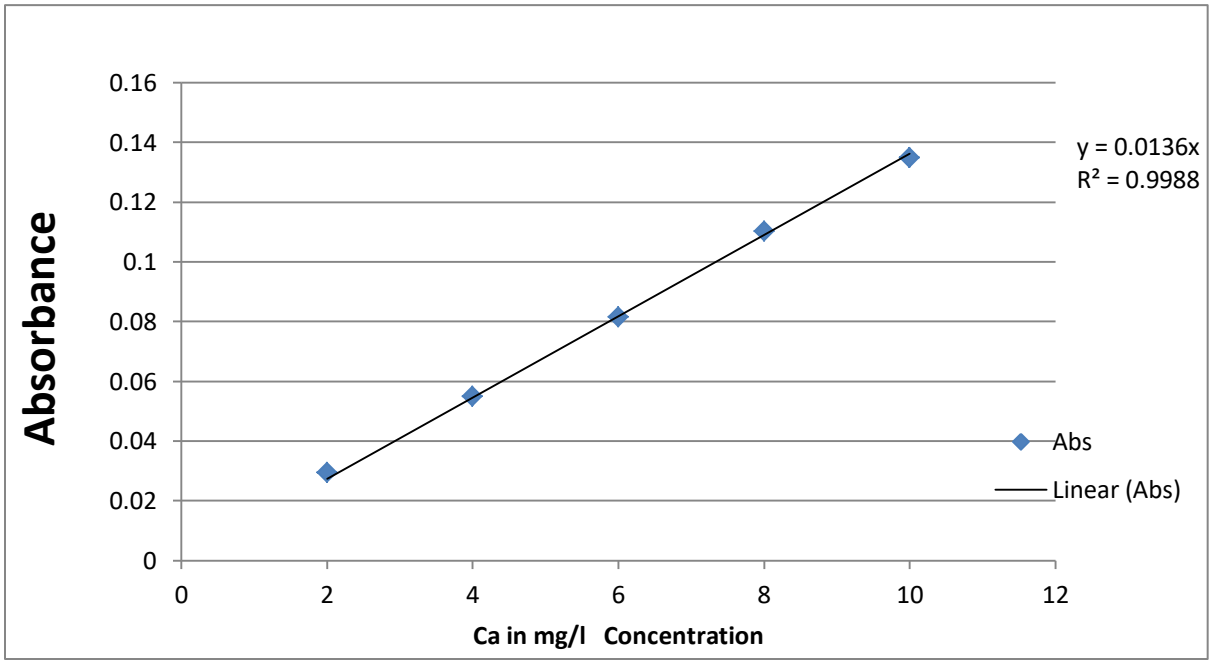
Appendix 7: ANOVA table for total coliform and *Staphylococcus aureus* of the cottage cheese

Parameter	Source	Sum of the squares	DF	Mean square	F value	Pr>F
Total coliform	Treatment	15.922	2	7.961	5.084	≤0.05
	Error	18.792	12	1.566		
	Total	34.714	14			
S.aureus	Treatment	17.609	2	8.804	40.092	≤0.001
	Error	2.635	12	0.220		
	Total	20.244	14			

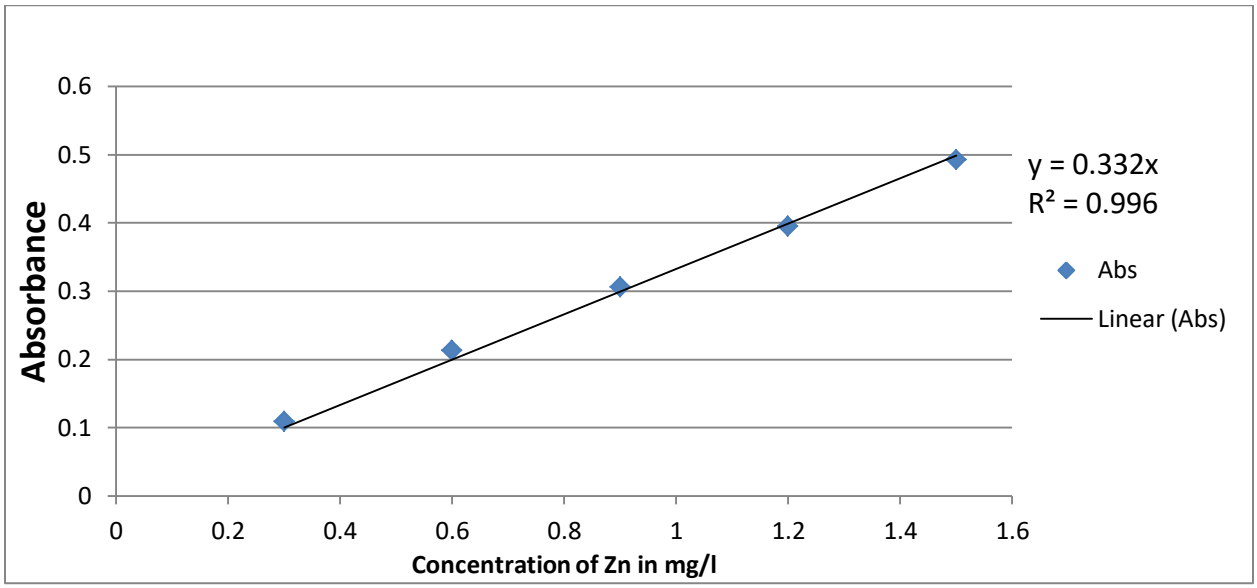
Appendix 8: Standard Calibration curve of Phosphorus



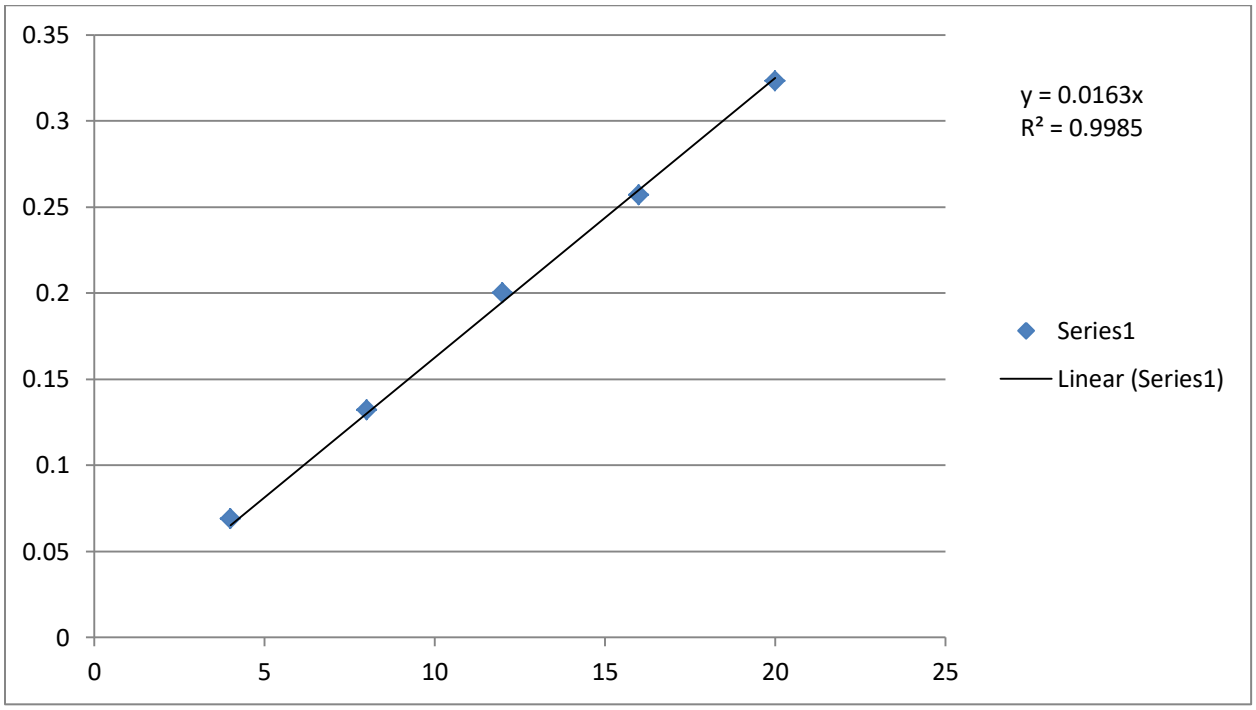
Appendix 9: Standard Calibration curve of Calcium



Appendix 10: Standard calibration curve of Zinc



Appendix 11: Standard Calibration curve for Iron (Fe)



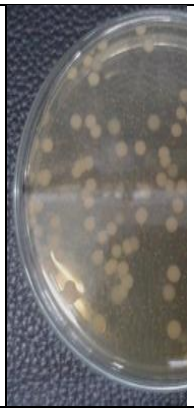
Appendix 12: Pictures during laboratory work

The left and right photos are positive results for clot-on boiling and alcohol tests respectively, while the middle photo is for the negative result of the clot-on boiling test.

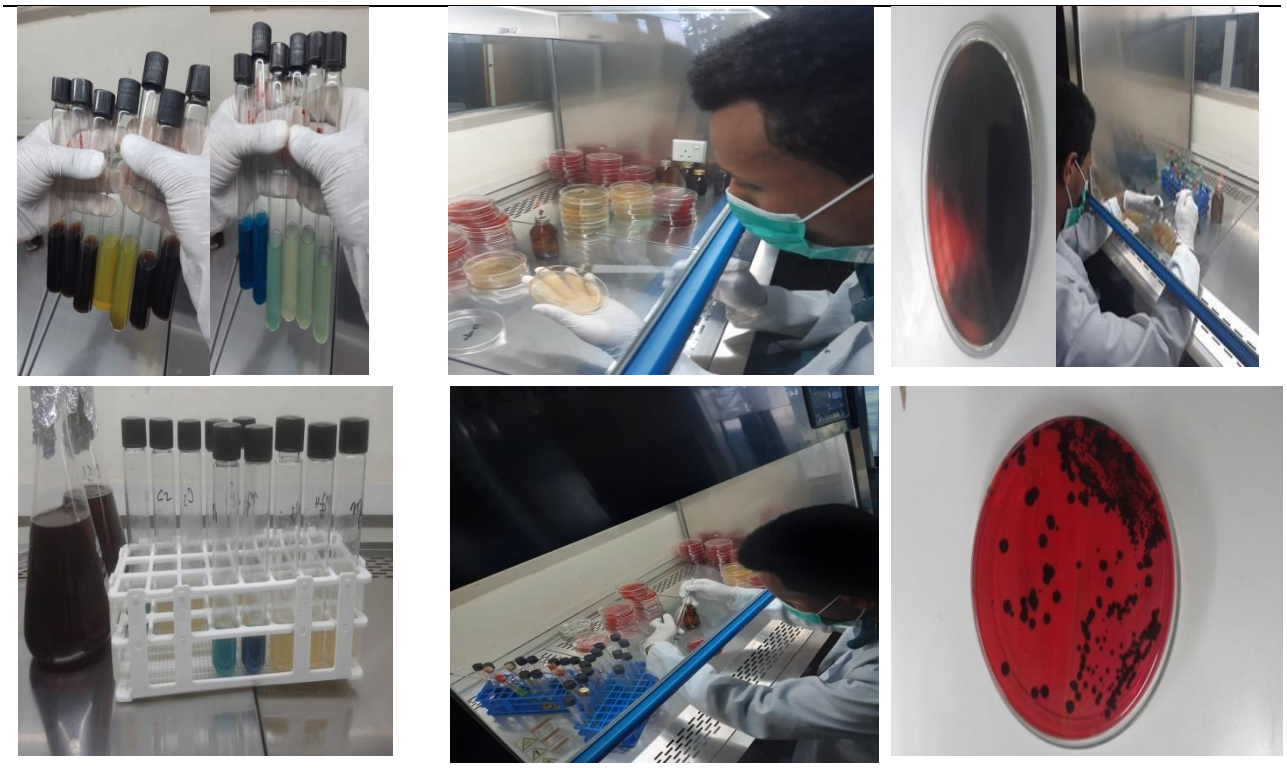

Physicochemical analysis of milk and cottage cheese *('ayib'*)


Mineral Analysis of milk and cottage cheese (*ayib*)

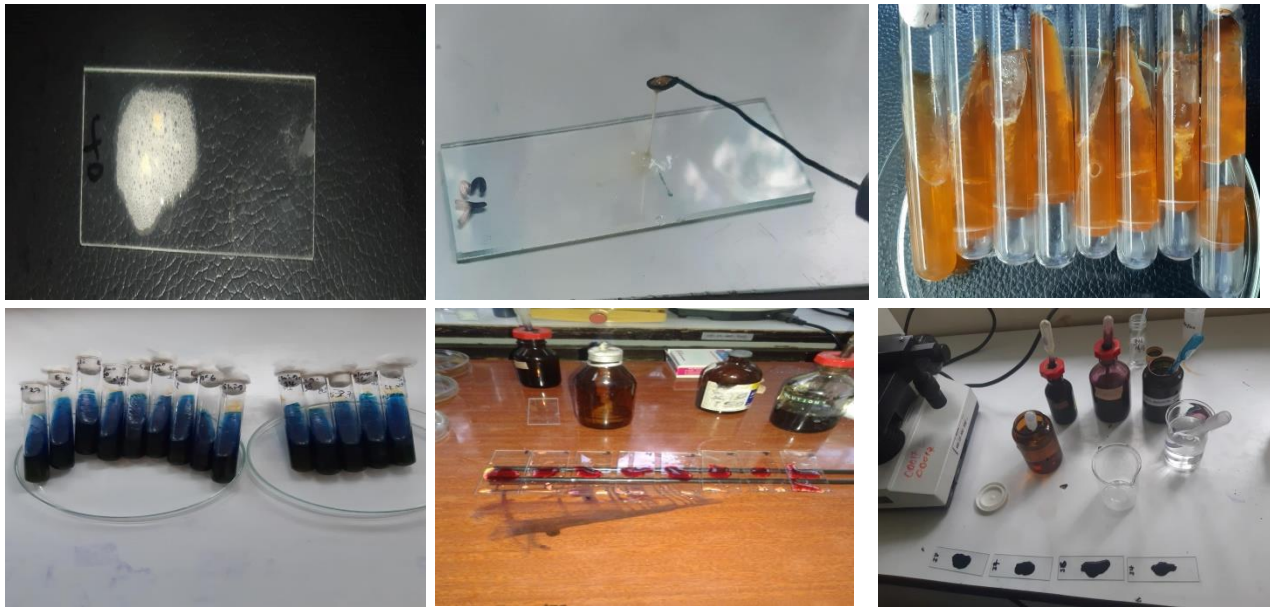





Enumeration of *Staphylococcus aureus* and total coliform from milk and cottage cheese



Isolation of Listeria and *Salmonella* Spp. from milk and cottage cheese



A series of biochemical tests for confirmation

Appendix 13: Survey questionnaires format

1. **Personal data**

Willingness of the respondent: _____ID of the respondent:_____ Address:_____

Name of Enumerator:_____ Contact address of Enumerator:_____

2. **Demographic Characteristic**

2.1 Sex of the respondent:

Male:_____ Female:_____

2.2 Marital Status of the respondent:

Single:_____ Married:_____ Divorced:_____

2.3 Age of the respondent:

18-30 years old:_____31-45 years old:_____ 46 Years and above:_____ prefer not to answer_____

2.4 Education status of the respondent:

Can read and write:____ Elementary school:___ High school:_____ Diploma/Degree _____Above:_____

2.5 Your Sub-City in Addis Ababa:

Arada:_____Yeka:_____Addis-Ketema_____Kolefe___

Gulale_____Bole:_____Kerkos: _____ Nefas _____ Lafto:_____ Akaki:_____

2.6 Your monthly income:

Full time employed _____Part-time employed: _____unemployed:_____ Self-employed:_____

3. **Dairy product availability and accessibility**

3.1 Where do you get the dairy products for your home?

Milk shop/supermarkets _____Food Restaurants: _____Open markets:_____ Distributors/retailer:_____ others:___

3.2 Are there milk shops/supermarkets or other dairy product suppliers around your home area?

Yes:_____ No:_____

3.3 The average distance from your home:

Nearly 200m:_____ Nearly 200-500m:_____ Nearly 500m-1Km _____ More than 1Km_____

3.4 Do you believe the distance can impact your dairy product consumption?

Yes_____ No:___

3.5 Cost of dairy products and your monthly income: Not at all:_____ Fair____ Very fairy_____:

4 Dairy product consumption habit

4.1 How often do you have dairy products at your home?

Daily:_____ Weekly: _____Bi-weekly:_____ Monthly:_____ Others_____

4.2 Do you consume dairy products across the year

Sure! Always: _____No! Only in fasting seasons: _____No! Only non-fasting season___

Occasionally in non-fasting seasons:_____

4.3 Do you expect dairy products are important for the children?

Yes:____ No:_____

4.4 Which ages of the children do you recommend more dairy product consumption?

Above 6 months- 2 year ____Above 6 months- 7 years:_____ Above 6 month-10 year:____

4.5 Do you/ your family include dairy products to the lunch of the children at schooling time?

Yes:_____ No:_____

4.6 Who prefer dairy products for the children for consumption?

Family:_____ Friends:_____ By themselves:_____ Relatives:_____ Others:_____

5. Nutritional knowledge, dairy products handling practices, and safety

5.1 Do you know the nutritional value of dairy products like whey?

Yes:____ No:_____

5.2 Do you see nutrition value while purchasing from shops/supermarkets?

Yes____ No:___

5.3 What is dairy product mostly consumed by pre-school children?

Boiled raw milk:____ Boiled pasteurized milk_____ Cheese/yoghurt and etc.:____

5.4 What is dairy product mostly consumed by adolescence?

Boiled raw milk:____ Boiled pasteurized milk:_____ Cheese/yoghurt and etc: _____

5.5 In the case of fluid milk is an unavailable or inadequate supply, what do you replace?

Egg and Animal Meat:_____ Milk powder:_____ Soya milk:_____

5.6 How do you store dairy products?

Refrigerator: ____Cold water: _____Upon using:____

5.7 How long do you store (days)

One of the day:_____ One day:_____ Two day:_____ More than two day:_____

5.8 Do you see the expiry date while purchasing from shops/supermarkets?

Yes: _____No____

5.9 What techniques you may use if no expire date labeled on packing materials?

Color:_____ Smell:_____ Taste:_____ I don't buy:_____ Others_____

5.10 Do you have any doubt on milk adulteration (water)? Yes:_____ No:_____