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# Non-nutritional Factors Affecting Milk Yield, Milk Quality and Prevalence of Food Borne Pathogens in Milk and Milk products in Central Highlands of Oromia, Ethiopia

**PhD Dissertation**

**By**

**Dereje Shibru Senbeta**

Addis Ababa University College of Veterinary Medicine and Agriculture
Department of Animal Production Studies
PhD Program in Animal Production

**IN FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ANIMAL PRODUCTION**

**April, 2020**

**Bishoftu, Ethiopia**

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# BIOGRAPHYICAL SKETCH

The author was born in South Western Shoa zone of Oromia National Regional State, Sebeta Awas District, at a place called *Meta Abo*, in June 1980. He attended his elementary and junior school at *Dima* elementary and Mulgeta Gedile Junior Secondary school, respectively, from 1989 to1995. He attended his secondary school in Sebeta Comprehensive Secondary School (SCSS) from 1996 to 1999.

After completion of his high school education, he joined Mekelle University to pursue his undergraduate studies. After four years of study at Mekelle University, he graduated with a BSc degree in Animal and Range science in July 2003. The author was then employed in Bekoji Agricultural TVET College and served as an instructor for three year, as college program cease, he was transferred to Gambella ATVET College. After serving for additional two years in the same College, he joined the School of Graduate Studies of Haramaya University and graduated his MSc studies in Animal Production in January 2012. Then after he served for one year in Gambella TVET College, he joined the then Mettu University, College of Agriculture and natural resource, Gambella campus as lecturer. One year later, he joined the School of Graduate studies of Addis Ababa University to pursue his PhD studies in Animal Production.

# DEDICATION

This dissertation is dedicated to my father Shibru Senbeta, Amare Lemma and my beloved wife Hawi Bulti, for their dedicated support and partnership in the success of my life.

# STATEMENT OF AUTHOR

First, I declare that this dissertation is my bonafide work and that all sources of materials used for this dissertation have been duly acknowledged. This dissertation has been submitted in partial fulfillment of the requirements for a PhD degree at the Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University Library to be made available to borrowers under rules of the Library. I solemnly declare that this dissertation is not submitted to any other academic institution anywhere for the award of any academic degree, diploma or certificate.

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# ABBREVIATIONS

| | |
|---|---|
| AMBC | Aerobic Mesophilic Bacterial Count |
| CSA | Central Statistics Agencey |
| EMBA | Eosin Methyl Blue Agar |
| FAO | Food and Agriculture Organization |
| FDA | Food and Drug Administration |
| GDP | Gross Domestic Product |
| GLM | General Linear Model |
| ICMSF | International Commission of Microbiological Specifications in Food |
| MPN | Most Probable Number |
| NZFSA | New Zealand Food Safety Authority |
| PMO | Pasteurized Milk Ordinance |
| QSAE | Quality and standard Authority of Ethiopia |
| TABC | Total Aerobic Bacterial Count |
| VRBA | Violet Red Bile Agar |
| WHO | World Health Organization |
| UHT | Ultra High Temperature |

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## ABSTRACT

*Nutritional and non nutritional factors are the major contributors for variation in milk yield and physicochemical parameters. On the other aspects as milk is nearly perfect food it is also a good growth media for spoilage and pathogenic microorganisms. This study was aimed at analyzing the effects of non-nutritional factors on milk yield and composition, physicochemical and microbial quality. The prevalence of some food borne pathogens in milk and its products was also evaluated in study areas. The study was conducted from, February 2017 to June 2019 in Holeta Agricultural Research Center (HARC) dairy Lab., milk and its product samples were collected from producers and collectors in Bishoftu and Sebeta town's while pasteurized milk was sampled from shops in Addis Ababa. The study was conducted in three categories where dairy farm recorded data analyis, physicochemical, microbial and food borne pathogenic investigations of milk and milk products samples collected from study sites. Twelve years recorded data of Holstein Friesian crossbred dairy cows were categorized and summarized into parameters of study to see the effects of season, exotic blood level, parity and lactation stages on monthly milk yield and composition from records of HARC dairy farm. Analysis of physicoche mical parameters of one hundred raw milk and twelve pasteurized milk samples collected from st udy areas were conducted using lacto scan in dairy laboratory of HARC. In addition to the retained one half amount of samples used in physicochemical analysis, samples of 40 locally fermented yoghurt and 40 cottage cheese(ayib) summed up to 192 samples were used for investigating microbial quality and food borne pathogens of milk and milk products from Sebeta and Bishoftu areas. The microbial quality and food borne pathogens analysis were done following standard procedures of laboratory in dairy laboratory of HARC. General linear model of SAS was used for statistical analysis of variances. Season significantly affected milk yield, fat and protein content of milk. Higher yield and fat percent composition was recorded in dry season while higher protein percent was recorded during wet season. Genotype significantly affected milk yield where 62.5% and ≥75% crossbred cows produced significantly higher milk yield than that of 50% crossbred cows. Differences in parity affected milk yield and protein content of milk where higher milk yield and protein content was recorded in parity five. Milk yield and protein content had shown increasing trend as dam parity advances. Mean monthly milk yield, percent of protein and total solid was varied significantly between diffe rent lactation stages where protein and total solid percentage was significantly higher in late sta* *ges of lactation. Differences in sample types were affected physical parameters of milk pH, specif ic gravity and freezing point.The overall result showed that percent composition of raw milk sam ple from Sebeta and Bishoftu area include: lactose (4.91±0.12)%, protein(3.28±0.08)%, fat(3.68 ±0.25)%, solid-not fat(8.93±0.22)%,total solids(12.61±0.41)%, ash(0.74±0.02)%; and lactose(4. 36±0.06)%, Protien(2.90±0.04)%, fat(3.59±0.13)%, solid-not-fat (7.93±0.11)%, total solids(11.5 2±0.20)%, ash (0.66±0.01)% respectively. Significant difference was observed in lactose, protein , and solid not fat, total solid and ash values between the study sites. Raw milk samples from Sebeta area had higher percentage of composition where as physicochemical components of pasteurized milk was significantly lower than that of raw milk and also below the minimum requirement of Ethiopian standard for protein, fat, total solid and specific gravity. Results of raw milk fulfill minimum requirements of standards to be accepted. There was significant variation due to difference in study site for TCC, E. coli, YMC, S.aureus and L.monocytogenes of raw milk samples but no variation in TABC due to differences in study sites on raw milk and milk products . There was also significant difference in S. aureus in cheese, TCC and E. coli in yoghurt due to difference in study site of sampling. Except E.coli, TCC, YMC, S.aureus and L. monocytogenes of raw milk samples were significantly higher in Bishoftu than sebeta milk samples.On the other ha nd, lower count of TABC, TCC, S. aurus and E.coli were found in pasteurized milk. There was high microbial count in milk and milk products of the two sampling site and sources where counts of the respective sites of TABC, TCC, yeast and mould (YMC), E.coli and L. monocytogenes were above Ethiopian quality standards. Given their high count, milk spoilage and health risk is inevitable if these products are consumed untreated. Pasteurized milk had showed less microbial loads, therefore it is one option recommended as solution for reducing the high microbial counts. The high L. monocytogenes in pasteurized milk suggested that the existence of either post process contamination or inefficient pasteurization. Hence, minimizing contamination through proper barn and equipment cleaning, animal health care, facilitating milk cooling systems, strict hygienic practices at farm, collection sites and in dairy processing plants are necessary.*

*Key words*: Season, parity, genotype, lactation stage, milk composition, microbial count, Foodborne pathogens

# 1. INTRODUCTION

Ethiopia possesses 60.39 million heads of cattles, 31.30 million sheeps, 32.74 million goats and 1.42 million camels (CSA, 2018). Among the cattle population: 54.68% are females and the remaining 45.32% are males (CSA, 2018). The livestock subsector has an enormous role to Ethiopia's national economy and livelihoods, contributing about 16.5% of the national Gross Domestic Product (GDP) and 45% of the agricultural GDP (FAO, 2019). It also contributes 10% of export earnings and 30% of agricultural employment (FAO, 2019; Behnke and Metaferia, 2011). The livestock subsector supports and sustains livelihoods of 31 to 48% households as an income source (FAO, 2019).

Despite the large cattle population, the prevailing favorable climatic conditions and resources for livestock production, the current milk production in the country is low. From about 12.39 million milking cows: the estimate of total cow milk production for the rural sedentary areas were about 3.32 billion liters (CSA, 2018) where as the estimate for total annual milk production is about 3.8 billion liters(FAO, 2019) and the estimate for camel milk is about 327.64 million liters (CSA, 2018).This is reflected by the low per capital milk consumption of about 20 kg(Azage, 2018, FAO, 2019) and increasing trend in imports of milk and milk products (Metaferia *et al*., 2011 and Getachew, 2003).

Milk is one of the most important food products with livestock origin which enjoys special significance in terms of its various nutritional properties such as protein, lactose, fat, minerals and vitamins. The composition of milk is also of the greatest importance for the process ability of milk in dairy industry. Milk protein can affect the coagulability state of it, together with the fat content it helps in quality studies of milk (Walstra *et al*., 1999). The milk components particularly protein and solid-not-fat based milk pricing scheme are adopted for the payment to milk producers in most developed countries (Cunha *et al*., 2010) placing a negative signal for those on volume base costs (Harding, 1995). Yet, milk is still marketed on volume basis with less emphasis on the milk components pricing in East Africa. However, recently, major dairy processors in the region have expressed strong interest in implementing a quality-based pricing system (Cheruiyot *et al*., 2018). Moreover, the growing consciousness on human health and changes in dietary habits introduce quality milk and milk product based pricing to suit the consumers' preferences. This induced dairy industry to produce diverse milk products through milk standardization and processing. However, prior to milk standardization, it is imperative to have basic empirical information on milk components and its seasonal variations. Studies in different countries reported that composition and amount of milk produced during the lactation period can be affected by feed, season and stage of lactation (Mackle *et al.,* 1999; Auldist *et al*., 1998; Sharma *et al*., 1985 and Hang *et al*., 1982).There was no enough studies with respect to this in Ethiopia therefore comprehensive information is needed to understand the effect of season and non-nutritional factors on yield and compositional quality of milk of cross-bred dairy cows.

On the other hand, in less developed countries especially in hot tropics, high quality and safe product is most important but not easily accomplished (DeGraaf *et al*., 1997). Improving quality is required since milk is a suitable substrate for microbial growth and repository of food borne pathogens (Enabulele *et al.,* 2014). Milk fluid (semi-fluid) nature and its chemical composition render it one of the ideal culture media for microbial growth and multiplication (Mogessie and Fekadu, 1994). Due to the highly perishable nature of milk and mishandling, the amount produced might be subjected to high post-harvest losses. In Ethiopia in earlier studies losses of up to 20–35% had been reported for milk and dairy products from milking to consumption (Getachew (2003). However recently, due to emerging modern retail sector there is by half reduction of average post harvest loses that has been observed in the traditional retail sectors. Berta *et al*. (2019) reported that the total post harvest losses over all segments of the value chain (farmer to retailer) in the most prevalent marketing channel of milk amount between 2.1 - 4.3% of total produced quantities. Beside either direct transfer of pathogens from the blood (systemic infection) of infected animal or from infection in the udder, microorganisms may contaminate at various stages of milk procurement, processing and distribution. The hot and humid climates of tropical for much of the year are ideal for quick milk deterioration as a result of growth and multiplication of many bacteria (Godefay and Molla, 2000). Therefore, safety of dairy products with respect to food-borne pathogens is of a great concern in developing countries particularly in Ethiopia,where production of milk and various dairy products take place under unsanitary conditi ons and poor production practices (Hawaz *et al*., 2015). Bacterial contamination of raw milk can originate from different sources such as air, milking equipment, feed, soil, faeces and grass

(Coorevits *et al*., 2008). Higher number of bacteria in raw milk can affect the quality, safety and consumer acceptance of products. Several human microbial pathogens such as *Listeria monocyto genes*, *Salmonella* spp., *Staphylococcus aureus* and *Mycobacterium tuberculosis* have been foun d to be associated with milk and milk products (Jayarao *et al*., 2006).

The presence of microorganisms in milk and milk products has important implications for safety, quality, regulations and public health. For example, high microbial counts in raw milk are responsible for quality defects in pasteurized milk, other processed dry milk, butter and cheese (Barbano *et al*., 2006).This means, milk with low bacterial counts results in higher quality milk products with increased shelf life (Raynal-Ljutovac *et al*., 2007). Additionally, selecting raw milk of high quality has been associated with a decrease in consumer complaints caused by fluid milk quality (Keefe and Elmoslemany, 2007). As a result, many countries have milk quality regulations which limits on the total number of bacteria in raw milk, to ensure the quality and safety of the final product. Hygienic quality control of milk and milk products in Ethiopia is not usually conducted on routine basis. There is limited information on the microbial quality of raw milk in Ethiopia (Korma *et al.,* 2018; Zelalem and Faye, 2006). Information on the bacterial content of a given milk sample may reflect on the state of health of the cow, the condition under which the milk is stored and distributed and its public health significance (Barbano *et al*., 2006).

Therefore, the aim of the study was to assess the effect of season, non-nutritional factors and source of milk samples for quality and safety of cow's milk and its products collected from the central Highlands of Oromia, Ethiopia.

The specific objectives of this study were:

- To evaluate the effect of season, stage of lactation, parity and exotic blood level, on milk yield and composition in the central Highlands of Ethiopia.
- To assess the handling practices of milk produced and marketed in the central highland of Oromia, Ethiopia.
- To analyze the physicochemical properties of raw and pasteurized milk produced and marketed in the central highland of Ethiopia.
- To investigate the microbial quality and prevalence of food borne pathogens in raw and pasteurized milk and processed milk products marketed in the study areas

# 2. LITERATURE REVIEW

## 2. 1 Effects of Season on Milk Yield and Composition

The measurable effects of environmental factors can be determined and used in the management of the farm (Cilek and Tekin, 2006). Environmental factors such as year of calving, season of calving and age at calving affect productivity (Bilgic and Alic, 2005). Many researchers (Kocak *et al.*, 2007; Erdem *et al.*, 2007) reported that the effect of calving season on 305 days milk yield was as significant and indicated that milk yield was higher in autumn and winter. Unlike, Bilgiç and Aliç (2005) and Pelister *et al.* (2000) reported that effect of calving season on 305 days milk yield was non-significant. Bormann *et al.* (2003) reported that the effect of calving season on milk yield was significant and milk yield was the highest in cows calving in winter. Similar findings have been reported by Javed *et al.* (2004) and Tekerli *et al.*(2008) in Holstein Friesian cows. Thorpe *et al.* (1993) showed the effects of season of calving on production performance of dairy cattle. Cows calving in winter have high milk yields, probably due to good feeding levels in the first 3 or 4 months of lactation. On the other hand, cows calving in summer have low milk yields due to their being subject to high environmental temperatures in the first 3 or 4 months of lactation. On the contrary many workers (Bilal, 2008 and Rege, 1992) observed that the season of calving had a non-significant effect on lactation milk yield in Holstein Friesian cows. According to Angel *et al.* (2013) cows that calved during the cold and dry seasons had similar milk yield per lactation. However, milk yield per lactation was 145 kg greater in cows that calved during the cold season compared to those that calved during the rainy season.

According to Jemila and Achenef (2012) season dramatically affects milk fat and protein. The hot, humid months depress fat and protein content. There is a gradual increase of protein and fat in milk through the fall and peak levels occur in the colder months of winter. As temperatures increase through the spring, component levels are gradually decreased. These changes may be indicative of feed intake patterns, which are lower in summer due to changes in weather and temperature. Milk yield traits and cow weight showed a significant increasing trend from first to fourth lactation. Milk yield was maximum during the cold and dry seasons and minimum during the rainy season, indicating that calving season also has a great influence on this trait.

Milk proteins and fatty acids are influenced by seasonal changes (Heck *et al*., 2009). The CP and Rumen degradable protein (RDP) intake of cows were higher in dry season compared to that of wet season due to shortage of quality and quantity of roughage feeds.

### 2.2 Effects of Parity on Milk Yield and Composition

According to Ángel *et al*. (2013) milk yield per lactation, milk yield per day, milk yield per calving interval, efficiency of milk production and cow weight at calving increased with increased lactation number but did not significantly differ between third- and fourth-lactation cows. Similarly cows of first lactation yielded less milk per lactation, per day. Cows of parity one was less efficient than second-, third- and fourth-lactation cows and older (Zewdu *et al*., 2013). Second-lactation cows yielded less milk per lactation, per day and per calving interval and were less efficient than cows of third and fourth lactations and older. In agreement with Angel findings Holstein Friesian cows reared under tropical conditions of Sudan, Gader *et al*. (2007) also found that third- and fourth-parity dams had greater milk yield per lactation and milk yield per day than first- and second-parity dams and that second parity dams had greater milk yield per lactation and milk yield per day than first-parity dams. Magalhães *et al.* (2006) and Guler *et al.* (2009), using Holstein Friesian cows, observed that the effect of lactation number on cumulative milk yield at 305 days and total test day milk yield showed an identical pattern to that on calving weight and milk yield traits evaluated. Palacios-Espinosa *et al*.(2001) observed higher milk production adjusted to 305 days as lactation number increased from one through three. On the contrary, Ngodigha and Etokeren (2009), working with crossbred cows of different Holstein Friesian inheritance (50, 75, 87.5 and 100%), found that first, second and third-parity dams yielded more milk than fourth parity dams. According to Ángel *et al*. (2013) finding dams of first, second, third and fourth parities did not differ in lactation length. Ahmed *et al*. (2004) reported that parity number did not affect lactation length in Local Zebu×Holstein Friesian, Local Zebu×Sindhi and Sahiwal×Holstein Friesian cows maintained in Bangladesh. In contrast, Gader *et al*. (2007) noted that first-, second- and third-parity dams had greater lactation lengths than fourth-parity dams and Kaya *et al*. (2003) reported that first-parity Holstein-Friesian dams showed longer lactations than second, third- and fourth-parity Holstein-Friesian dams. Zewdu *et al*. (2013) reported that the lactation milk yield was significantly affected by parity hence, first lactation cows had lowest milk production, and highest production in 5$^{th}$parity. Milk proteins and fatty acids are influenced by parity (age) (Ng-Kwai-Hang *et al.*, 1987)

### 2.3 Effects of Genotype on Milk Yield and Composition

Cattle in the tropics have, on average, lower milk yields and shorter lactations than dairy cattle in temperate countries; the difference is caused by both genetic and non-genetic factors (Rege, 1998).One of the most common ways of increasing dairy production in the tropics and subtropics is through importation of breeds with superior genetic potential from other countries either for use in purebred breeding or in crossbreeding with local breeds (McDowell, 1985). Holstein Friesian and Jersey cross breeds display very different performance characteristics, with a notably higher milk yield in the Holstein breed compared with a higher milk nutrient density and a lower body weight in the Jersey breed (Capper and Candy, 2012). Although the largest reason for the breeds' milk production differences is their actual breed, the production system their involved in can also make a substantial difference in their production, components and yield. Management factors contribute to about 45% of the variation in milk composition and genetics explaining 55% (Van Tassell *et al.,* 1999). This means that the biggest factor in the animal's milk components is her genetics and breed, even though management plays a big role. Hence, recognition of high production of the Holstein Friesian breed has stimulated interest in setting up dairy industries in tropical and subtropical countries (Abubakar *et al*.,1986). In agreement with th is Aynalem *et.al*.(2008) reported that the fat, protein, SNF and TS percentages of 50 and 62.5% Holstein Friesian crosses were higher than those of 75% and 87.5% genetic groups. The 50% and 62.5% genetic groups did not differ in milk constituents. Moreover there is an indication that as the exotic gene level increased the percentages of the milk constituents declined significantly. In comparison of Jersey and Holstein Friesian milk for cheese production, Jersey come out on top as they test higher in fat % and protein % which amounts to less input for a greater output (Capper and Cady, 2012). Milk proteins and fatty acids are influenced by genetic variations (Gaunt, 1973).

## 2.4 Effects of Lactation Stage on Milk Yield and Composition

In the early stage of lactation cows usually experience negative energy balance due to their inability to meet daily energy requirements (Kaya *et al*., 2003). During this phase cows tend to convert adipose tissue into milk fatty acids. Short chain fatty acids are usually higher at this particular phase compared to long chain fatty acids. As lactation progresses inhibition of the de novo enzymes is lifted leading to production of more short and medium chain Fatty acid (FA) (Samkova *et al*., 2012).

Mech *et al*.(2008) reported that there is a peak milk yield at the mid of lactation which agrees with the stated milk yield increased up to 90 days and remain high for awhile and then declines in late stage of lactation. In agreement with this Melaku and Gurmessa (2012) also reported that highest milk yield was recorded in mid stage and lowest in late stage of lactation whereas fat content of the milk was significantly higher in early and late than mid stage of lactation. According to Akers (2002) finding Milk yield was significantly affected by pregnancy where pregnancy has a negative effect on milk yield. This may be due to hormonal changes, causing regression of the mammary gland and nutrient requirements of the fetus, reducing available nutrients for milk production (Bell *et al*., 1995). Similarly Bhosale *et al*. (2009) reported that fat content was lowest in mid lactation and significantly increased in early and late lactation stages. Contrary to this Bohmanova *et al*.(2009) reported that fat content of the milk was lower especially in late stage of lactation.

Milk proteins and fatty acids are influenced by lactation stage (Ng-Kwai-Hang *et al*., 1987). Higher protein content of pregnant animal milk may be related with the general increments in anabolism of major nutrients. Equivalent to this finding Casoli *et al*.(1989) and Dell'Aquila *et al*.(1993) were also observed in sheep. However, SNF and protein contents of milk were not significantly affected by stage of lactation and parity. Pollott (2004) reported that the lactose content of the milk was affected significantly by pregnancy only; other factors didn’t affect its composition in milk. Because there is a close relationship between lactose synthesis and the amount of water drawn into milk makes lactose a stable milk component.

### 2.5 Hygienic Practices and Physicochemical Properties of Raw and Pasteurized Milk

#### 2.5.1 Hygienic practices for milk production

Maintaining the sanitary condition of barn, milking, hygienic handling of milk equipment and milk after milking is important for the production of good quality milk. Proper and clean housing environment is a prerequisite to produce milk and milk products of acceptable quality (Asaminew, 2007). Clean, dry and comfortable bedding condition is important to minimize the growth of pathogenic microorganisms (Gurmessa, 2015). In most study in Ethiopia plastic containers were the mnost commonly used milking, milk collecting and storing materials above 90% respondents of Jimma(Duguma and Geert, 2015) and that of Bishoftu (Kebede and Megerrsa, 2018) report assure this. Respondents in most parts of Ethiopia clean milk handling containers before and after use. Quinn *et al.* (2002) reported that cooling milk after milking reduces risk of the growth of both pathogenic and spoilage bacteria. But in most study results of Ethiopia, farmers did not practice milk cooling after milking, because of lack of facilities for cooling milk, which is a serious problem to hygienic milk production.However there were reported findings where 50% of the farmers in Wolayta Sodo cooled milk immediately after milking (Benta and Abtamu, 2011). In the barn udder of the milking cows may have direct contact with the ground, urine, dung and feed refusals, therefore cleaning the udder of cows before milking is important hygienic practices required to ensure clean milk production (Zelalem *et al.*, 2011; O'connor, 1995)

#### 2.5.2 pH value

Milk pH gives an indication of milk hygienic and it should not be ≤6.6 or ≥6.8 when milk temperature is 20$^{o}$C, because cooling of milk reduces the risk of growth of bacteria while high milk temperature must be considered as favourable to the growth of bacteria in milk (Walstra *et al.,* 1999). The pH values higher than 6.8 indicates mastitic milk and pH values below 6.6 indicates increased acidity of milk due to bacterial multiplication (O'Connor, 1995). The titratable acidity test measures the acidity of the milk. Both pH and titratable acidity (TA) are measures of acid. TA is a more reliable indicator because relative to pH measurement, it is more sensitive to small changes in milk acidity, especially important in cheese making. The acidity of milk is of two types; natural acidity due to citrates and phosphates present in the milk and dissolved $CO_2$ during the processing of milking. The second is the developed acidity due to lactic acid produced by bacteria using the lactose in the milk as a nutrient, converting it to lactic acid. The acidity of milk measures the total acidity (natural acidity of milk and developed acidity). The International Standard Method for titratable acid is (ISO 6091, 2010). Milk normally exhibits an initial acidity of 0.14 to 0.16% when titrated using sodium hydroxide to a phenolphthalein end-point (O'Connor, 1995).

### 2.5.3 Specific gravity

Specific gravity is the ratio of density of the substance to the density of standard substance (water). Therefore, the specific gravity of milk is the ratio of the density of milk to density of water. The reading is done using a lactometer. The density of a substance varies with temperature, it is necessary to specify the temperature when reporting specific gravities or densities. The specific gravity of milk is influenced by the proportion of its constituents (e.g. composition), each of which has different specific gravity approximately as follows; water (1.000), fat (0.930), protein (1.346), lactose (1.666), salts (4.12) and SNF (1.616). The specific gravity of milk is decreased by addition of water, addition of cream (fat), while removal of fat, addition of skim milk and reduction of temperature increase specific gravity of milk. Generally, normally milk has a specific gravity between 1.027 and 1.035 with an average value of 1.032 at $16^{\circ}C$ (O'Connor, 1994, Morris, 1999; Draft East African Dairy Standards on raw milk, 2010).

### 2.5.4 Freezing point

This is the most constant physical property of milk and is determined by the number of solute particles present. Milk freezes at a lower temperature than normal water with its freezing point lying between -0.525ºC and-0.565ºC. Presence of soluble constituents lowers the freezing point. The freezing temperature can be determined using a cryostat (Draft East African standards on raw milk, 2010) and also through the use of electronic milk testing devices e.g. the lactoscan (La ctoscan website, http://www.lactoscan.com/usefull-info/english/freezepoint.html). Added water can be measured by changes in the freezing point of milk from its normal values, the current official freezing point limit is−0.525° Horvet or−0.505°C and was designed for whole-herd, bulk-tank samples, or processed milk samples. The freezing point of milk is the constant physical-chemical property of milk, which is determined only by its water soluble components such as lactose, and salts, which in accordance with the Wigner law are held in milk at an approximately constant concentration. However, the mineral composition of milk depends on lactation, nutritional status of the animal, and environmental and genetic factors (Zamberlin *et al.*, 2012). Adulteration of milk with water will cause a measureable rise of the freezing point of milk. The freezing point is also lowered by acidification of milk, which leads to protein denaturation. The freezing point is considered as an accurate and sensitive method, most laboratories use a cryoscopy, method that is the ISO reference method (ISO 5764:2009).

### 2.5.5 Chemical or nutritional composition of milk

Chemical composition of milk is highly variable and influenced by intrinsic factors like breed, species, stage of lactation, external factors like environmental stress, changes in feeding, *etc*. Milk composition and production are the interaction of many elements within the cow and her external environments (O'Connor, 1995). However, it is generally accepted that the dairyman can alter many of these factors to achieve milk production and increase profit. The major milk components include water, proteins, fat, lactose (milk sugar), minerals and other minor components such as vitamins and enzymes (Eckles *et al.,* 1980). All the constituents other than water are termed as Total Solids (TS), while the total solids minus the fat contents give the Solids-Not-Fat (SNF). Water contents vary from 85.5 to 89.5%, total solid from 10.5-14.5%, fat from 2.5 to 6.0%, solid-not fat from 8.0 to 8.5%, proteins from 2.9 to 5.0%, lactose from 3.6 to 5.5% and minerals from 0.6 to 0.9% (O'Connor, 1995). The composition of milk determines the quality and quantity of dairy products processed.

The study conducted by Workneh (1997) and Alganesh (2002) indicated that the content of cows' milk fat ranges from 2.5 to 8%. The local Zebu cattle milk contains more fat (5.6%) as compared to most exotic breeds (O'Connor, 1995). Kiwuwa *et al.* (1983) also reported higher milk fat percentage in Zebu compared to crossbreds (5.3 vs. 4.4%). Study conducted by Helen

(2007) showed that the average composition of cows' milk in Kombolcha woreda of eastern Hararghe was 3.67%, 6.08%, 16.77%, 10.70% and 0.92% for protein, fat, TS, SNF and ash, respectively. Other authors also reported that protein content of cows' milk ranges from 2.9 to 5 and that of total solids ranges from 10.5 to 14.5% (Workneh, 1997; Alganesh, 2002).

Table 1 Comparison between nutrient contents of raw milk and pasteurized milk of Ethiopian , FAO and European Union (EU) quality standards

| Components | Ethiopia(ES,2009)% | (FAO,1988)% | EU standards (Raff, 2011)% | Pasteurized milk (ES)% |
|---|---|---|---|---|
| pH | NA | 6.6-6.7 | NA | NA |
| Sp.gravity | NA | NA | NA | NA |
| Freezing pts | -0.525 --0.550 | -0.54°c | > –0.515 °C | -0.525 to --0.550 |
| Lactose | NA | NA | 4.2 | NA |
| protein | 3.2 | 3.5 | 2.73 | 3.2 |
| Fat | 3.5 | 3.7 | 3.25 | 1.5-3.5 |
| SNF | 8.30 | 8.5 | 8.25 | NA |
| TS | 12.8 | 13.47 | 12.5 | 12.8 |

Source FAO, 1988; ES, 2009; Raff, 2011, NA=Not Available

### 2.6 Microbiological Quality of Cow Milk

Improving the microbial safety of perishable foods is currently a major pre-occupation in the food industry (Vachon *et al.*, 2002). On-farm cooling and hygienic practices are critical, with any failure adversely impacting the microbial load in raw milk. Correct sanitizing procedures for packaging and effective cooling management practices for the raw milk are important steps for minimizing cross contamination and growth of any microorganism present in the raw milk (ICMSF, 1996). Rinsing of milking machine and milking equipment with unclear water may also be one of the reasons for the presence of a higher number of microorganisms including pathogens in raw milk (Bramley and McKinnon, 1990)

Milk from healthy cows contains relatively few bacteria and the health risk from drinking raw milk would be minimal. However, milk is a natural food that has no protection from external contamination and can be contaminated easily when it is separated from the cow (Rosenthal, 1991). Raw milk has a high water activity (aw =0.99) and an almost neutral pH (Roos, 2011). Milk is an excellent substrate for the growth of many microorganisms (ICMSF, 2005) especially pathogenic microbes.Thus, the quality control of milk is considered essential to the health and welfare of a community. The first of these measures involves efficient cooling of milk to 4°C immediately following milking. The temperature of freshly drawn milk is about 38°C. Bacteria multiply very rapidly in warm milk and milk sours rapidly if held at these temperatures. Reduced temperatures inhibit growth of mesophils and thermophils and reduce the activity of degradative enzymes. But if is not cooled and is simply stored in the shade at an average air temperature of 16°C, the temperature of the milk will only have fallen to 28°C after 3 hours. Cooling the milk with running water will reduce the temperature to 16°C after 1 hour. At this temperature bacterial growth will be reduced and enzyme activity retarded. Thus, milk will be kept longer if cooled. Modern dairy farms use refrigerated bulk storage tanks which maintain milk at 4°C or below (Marth and Steele, 2001).

The numbers and types of microorganisms in milk immediately after production (i.e. the initial micro flora reflects directly microbial contamination during production) enables to assess the quality of milk. The microflora in milk when it leaves the farm is determined by the temperature to which it has been cooled and the temperature at which it has been stored (O'Connor, 1995). Milk is cooled to and stored at $\leq 4^{\circ}C$; the low temperature will normally prevent bacterial multiplication for at least 24 h and the micro flora is, therefore, similar to that present initially (Robinson, 1990). Although milk produced from the mammary glands of healthy animals is initially assumed to be sterile but microorganisms are able to enter the udder through the teat duct opening.

### 2.6.1 Total aerobic bacterial count of cow milk

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. The microbial content of milk is a major feature in determining its quality and the types of micro-organisms in milk immediately after milking are affected by factors such as equipment cleanliness, season, feed and animal health (Rogelj, 2003). Bacterial contamination of raw milk can originate from different sources such as air, milking equipment, feed, soil, faeces and grass (Coorevits *et al*., 2008).

The total bacterial count refers to all viable microorganisms that could grow aerobically and form countable colonies on plate count agar incubated at 35ºC for 48hrs (Jay, 2000). It is the bacterial growth per ml of milk over a fixed period of time (Blowey and Edmondson, 1995). Milk with high TABC should be avoided since some bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Streptococcus agalactiae*) found in raw milk can cause diarrhoeal disease and food poisoning (Gilmour and Rowe, 1990). The initial Aerobic Mesophilic Bacterial Count (AMBC) values in milk, for example >1 x $10^5$cfu/ml, are evidence of serious faults in production hygiene, where as the production of milk having AMBC values< 20, 000cfu/ml reflects good hygienic practices. The bacterial load in fresh raw milk should be less than 50,000cfu/ml when it reaches the collection point or processing plant (Pandey and Voskuil, 2011). According to Shunda *et al*. (2013) the mean AMBC value of 180 samples collected from dairy farms, vending shops and homes/cafeterias in Mekelle was 7.35±0.180, 7.35±0.180 and 7.42±0.272 log cfu/ml, respectively. A total of 40 fresh whole milk samples that collected in and around Boditti town, Southern Ethiopia and the mean value of total AMBC was 6.36±0.24 log cfu/ml (Asrat *et al*., 2012). A total of 40 raw milk samples were also tested for AMBC from producers of two agro-ecologies(Dega and Woina Dega) in Ezha district of the Gurage zone, Southern Ethiopia and the value was 9.82 log cfu/ml (Abebe *et al*., 2012). A total of 78 milk samples were also collected directly from the udder, storage containers at farm level (bulk) and distribution containers upon arrival at selling points in Hawassa, Southern Ethiopia and the overall mean value of AMBC was 4.57, 7.28 and 10.28 log cfu ml-1, respectively (Haile *et al*., 2012). On the other hand, 100 samples collected in Jimma, Western Ethiopia showed that mean count of 7.5±0.8 and 6.06±0.6 log cfu ml-1for AMBC and lactic acid bacteria, respectively (Alebel *et al*., 2013). Milk samples were also collected in Jimma from 47dairy herds 4 times and the mean value of total AMBC was 9.62×105 cfu/ml (Tadele, 2013).

The standard plate count is suitable for estimating bacterial population in most types of dairy products and it is the reference method specified in the Grade A Pasteurized Milk Ordinance to be used to examine raw and pasteurized milk and milk products (Marshall, 1992). The European

Union (EU) had set standards for raw milk offered for sale. The basic milk hygiene requirement in the EU for total aerobic bacterial count should be less than$10^5$ cfu /ml and in many ways the directives are comparable to the US “Grade A” Pasteurized Milk Ordinance (Hillerton and Berry, 2004).

**2.6.2 Coliform counts**

Coliforms are a group of bacteria that comprise all aerobic and facultative anaerobic, gram-negative, non-spore-forming rods able to ferment lactose and produce acid and gas at 35$^o$C within 48 hrs (Jay, 2000). Coliforms, and in particular Escherichia coli, are regarded as indicators of recent faecal contamination if they are found in water, since they die out rapidly in water, but in most dairy products they do not die out, and the conditions are favorable to their growth. Coliforms are referred to as indicator microorganisms, since their presence is used to indicate the potential presence of pathogens in foods (Britz and Robinson, 2008). Micro-organisms occur in the air and in dust particles originating from manure, soil and feed. Conditions that increase the dust content in the air around the milking area will increase the microbial population and lead to increased bacterial contamination of the milk particularly where hand milking is practiced.

The detection of coliform bacteria and high microbial count in milk are major factors in determining its quality. It indicates the hygienic level exercised during milking, that is, cleanliness of the milking utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animal (Spreer, 1998; Gandiya, 2001). Milk from a healthy udder contains few bacteria but it picks up many bacteria from the time it leaves the teat of the cow until it is used for further processing. Coliforms are indicators of both the manner of handling milk from milking till consumption and the quality of the milk.

European Union standards and UK hygiene and food safety regulation recommended TCC for raw cow’s milk for direct consumption to be <100 cfu/ml (Fernandes, 2009; Hickey, 2009) whereas East African Community standards (EACS) (2006) for good quality raw cow milk TCC should not exceed mean of 3log10 cfu/ml.

### 2.6.3 Escherichia coli

*E. coli* is a rod-shaped member of the coliform group which is distinguished from most other coliforms by its ability to ferment lactose at 44°C. It is one of the hygiene indicator organisms" refers to the selected surrogate markers. The main objective of using bacteria as indicators is to reflect the hygienic quality of food. Escherichia coli strains conform to the general characteristics described for coliform groups. Most strains of Escherichia coli are normal inhabitants of the gastrointestinal tract of humans and animals.

*E. coli* is frequently a contaminating organism compared to other microbes and it is a reliable indicator of fecal contamination (Kumar and Prasad, 2010). Escherichia coli presence in foods is considered an indication of direct or indirect fecal contamination. Direct fecal contamination occurs during the processing of raw foods of animal origin, and because of poor personal hygiene of food handlers. Indirect contamination can occur through sewage and polluted water (FDA, 2002). Substantial number of E. coli in food suggests a general lack of cleanliness in handling, improper storage directive to possible presence of enteric pathogens in food with the considerations that they are nonpathogenic and occur in gastro intestinal tract as commensals in high numbers. Most *E. coli* are harmless, but a substantial population is known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man (Kaper *et al.,* 2004, Kumar and Prasad, 2010). Pathogenic Escherichia coli are characterized into specific groups based on vi rulence properties, mechanisms of pathogenicity and clinical syndromes (Doyle *et al*., 1997). Th ese groups include enteropathogenic E.coli, *enterotoxigenic Escherichia coli*, *enteroinvasive Escherichia coli*, *entero aggregative Escherichia coli* and *enterohaemorrhagic Escherichia coli* (EHEC). Escherichia coli natural habitats are the intestines of cattle, which creates the potential for contamination of milk and dairy products. In spite of this risk, milk and dairy products have been only occasionally implicated in out breaks of *Escherichia coli* food poisoning and even more rarely does an outbreak involve a pasteurized product (Kirk and Rowe, 1999). A survey carried out in UK for 12 month period looked at 1674 samples taken from 60 dairy farms and 714 raw milk producers were reported E. coli presence in 62% of milk samples tested (Rampling, 2000).

### 2. 6.4 Yeasts and moulds

Yeasts and molds commonly associated with milk and milk products are: *Saccharomyces* spp. /*Kluyveromyces* spp., *Candida* spp., *Torulopsis* spp.; and *Penicillium* spp., *Rhizophus* spp., *Aspergillus* spp., *Geotrichum Candidum*, *Alternaria* spp., *Cladosporium* spp., respectively (Vishweshwar and Krishnaiah, 2005). The overall mean of yeast and mold count for a total of 78 milk samples collected directly from the udder, storage containers at farm level (bulk) and distribution containers upon arrival at selling points in Hawassa was 3.03, 4.65 and 7.13 log cfu ml-1, respectively (Haile *et al.*, 2012). Mean count of yeast and molds was reported as 5.1±0.5 and 3.7±0.6 log cfu/ ml, respectively from 100 milk samples collected in Jimma (Alebel *et al.*, 2013). A total of 100 raw cow milk samples, 88 from individual farmers and 12 dairy farms, were also collected in Jimma and the overall mean counts of yeast and molds was 4.9±0.6 and 4.7±0.52log cfu/ml and 4.61±0.5 and 4.09±0.2352log cfu/ml, respectively (Tadesse and Bacha, 2014). Yeast and molds may be found as part of the normal flora of a food product on inadequately sanitized equipment or as airborne contaminants. Different groups of fungi are found in soil, barn dust, feeds, manure, and unclean utensils. They can produce toxic metabolites, resistance to freezing environments, and cause off odors and off flavors of foods (Herrera, 2001) and, which can spoil/reduce shelf life of milk and may also pose serious health problems to the consumer.

### 2.6.5 Pathogenic microorganisms in cow milk

The primary cause of a high somatic cell count in milk is intra mammary infection.The majority of bovine intra mammary infections are caused by bacteria. Many of these bacteria are also the causative agents of human diseases, these includes *Escherichia coli, Staphylococcus aureus, Salmonella and and Listeria Monocytogenes* and the likes (Leigh, 2005; CDC,1995).

#### 2.6.5.1 Salmonella

Salmonella species are facultative anaerobic, gram-negative, straight, small (0.7-1.5 x 2.0 -5.0 µm) rods, which are usually motile with *Peritrichous flagella* (Chris and Alec, 2002). The minimum pH at which Salmonella spp. can grow is dependent on the temperature of incubation, presence of salt and nitrite and the type of acid present. However, growth can usually occur between pH 3.8–9.5 (Jay *et al*., 2003) with the optimum pH ranging from 7.0 –7.5 (ICMSF, 1996). In addition, Salmonella grows best between 35 and 37°C but can grow between 7 and 49.5°C. Although water activity has a significant effect on the growth of Salmonella spp., with the lower limit for growth being 0.94, it can remarkably survive for months in dry environments and some sugary food items such as chocolate at a water activity of 0.3 to 0.5.

They are widely distributed in the nature with humans and animals being their primary reservoirs. Wide ranges of contaminated foods are associated with salmonella food poisoning including raw meats, milk and dairy products (Forsythe, 2000). Its food poisonings results from ingestion of food containing appropriate strains of this genus in a significant numbers. Salmonella primary habitat is intestinal tract of the animals where the organisms are excreted in feces from which they may be transmitted by insects and other living creatures to large number of places (Radiostits *et al*., 2007). Report of Jay *et al*. (2003), stated that pasteurization of dairy products effectively inactivates Salmonella spp., however contamination of milk may occur due to improper pasteurization and or post-processing contamination. According to the reports of Casemore (1987) in the UK and elsewhere milk has long been recognized as a source of *salmonella* infection (salmonellosis), which can be serious with systemic involvement and sometimes death, especially among the very young or elderly. On the other hand, the reports of FAO/WHO (2002) indicated that the outcomes of exposure to *Salmonella spp.* can range from having no effect, to colonization of the gastrointestinal tract without symptoms of illness or colonization with the typical symptoms of acute gastroenteritis. Jayarao and Henning (2001) examined bulk tank milk from 131 dairy herds' salmonella spp. was detected in 6.1% of the samples. Moreover, Jayarao *et al*. (2006) examined in bulk tank milk from 248 participating dairy herds for food borne pathogens. *Salmonella* was detected in 6% of the samples tested.

#### 2.6.5.2 Staphylococcus aureus

Staphylococcus aureus are gram-positive, facultatively anaerobic, non-spore forming cocci. This pathogen produces a wide range of pathogenicity and virulence factors like staphylokinase, hyaluronidases, coagulases and haemolysins (Forsythe, 2000). Although several bacterial pathogens can cause mastitis, *Staphylococcus aureus* is one of the most important agents in mastitis of cows (Mørk *et al.*, 2005). Moreover, Miles *et al.* (1992) described that *Staphylococcus aureus* is probably the most infectious agent as it causes a chronic and deep infection in the mammary glands that is extremely difficult to cure. The Staphylococcus aureus bacteria enter the udder through the teat duct, and able to colonize the duct itself and frequently associated with subclinical mastitis leading to milk contamination (Jablonsky and Bohach, 1997). Staphylococcal food poisoning is caused by the ingestion of food containing pre-formed toxins, named enterotoxins secreted by this pathogen. Acording to Ibtisam and El Owni (2009) who reported that In raw milk, *Staphylococcus aureus* was detected in 26.66% of the 249 samples collected from farms and milk vendor points (and 41% of isolate from 644 raw milk sample by Suliman and Mohamed (2010). Mennane *et al.*(2007) Finding also reported that from 36 raw milk samples tested staphylococcus aureus was found at an average of $1.2x10^6$cfu/ml. Furthermore, survey studies had also revealed that *Staphylococcus aureus* was involved in 15% of recorded food borne illnesses caused by dairy products in eight developed countries (DeBuyser *et al.*, 2001). According to the same report, *Staphylococcus aureus* was responsible for more than 85% of the dairy borne diseases in France. It has the ability to produce several enterotoxins that are responsible for food poisoning. The type of food poisoning caused by *Staphylococcus aureus* is characterized by nausea, vomiting, and abdominal cramps, often with diarrhoea but without fever. The onset of the symptoms is rapid, often appearing 1- 6 h after ingestion of the contaminated food (Cliver, 1990). The temperature range for the bacterium to form toxin is from 10 to 45°C and optimal at around 35 to 40°C. Hence, normal refrigeration temperature can restrict the formation of toxin or they are likely to survive but are not usually able to grow, and their presence may be a cause of concern for health (Fernandes, 2009). On the other hand, S. aureus is a salt-tolerant microorganism and grows at a water activity as low as 0.85 which corresponds to a salt content around 25% w/w. Hence, it may grow better than the other bacteria in salt-containing products or products with low water activities (NZFSA, 2001). Even though most cases of infection are caused by S. aureus, other coagulase-positive Staphylococcus species, such as S. intermedius can also produce enterotoxins that cause food poisoning (Health Protection Agency, 2009). The bacterium can be destroyed by normal cooking procedures or pasteurisation, while the toxins produced are more resistant to heat; they may survive in food causing food poisoning (NZFSA, 2001).

#### 2.6.5.3 Listeria monocytogenes

Listeria monocytogenes is the most important pathogen with respect to human health. L. monocytogenes is naturally present throughout the environment and is commonly found in soil and on vegetation. The main route of transmission of *L. monocytogenes* to humans is via food, according to a New Zealand expert elicitation process (Cressey and Lake, 2007).

Listeria monocytogenes is of paramount significance in human and veterinary health (Kalorey *et al.*, 2008). The most common clinical form in cattle is encephalitis, in general, small numbers being affected (8-10% of the herd) with the animals surviving from 4–14 days. In animals, susceptibility to infection with L. monocytogenes has been attributed to decreased cell-mediated immunity associated with advanced pregnancy (Quinn *et al.*, 2002). L. monocytogenes has the ability to invade both phagocytic and non-phagocytic cells. L. monocytogenes causes two forms of listeriosis: Invasive listeriosis (usually just called listeriosis) and non-invasive gastrointestinal listeriosis (also called febrile gastroenteritis)(king *et al.*,2014).Human acquisition of listeriosis from animal sources has been shown to occur as an occupational hazard especially in farmers, butchers, poultry workers and veterinary surgeons. On the other hand, most human infections are food borne and the association of contaminated milk and dairy produce consumption with human listeriosis has been highlighted (Schlech, 2000).Starting in the 1960s, as a result of the introduction and wide spread use of refrigerators, processed foods and extended shelf life foods became more associated with listeriosis due to L. monocytogenes (Lamont *et al.*, 2011).This pathogen in addition to its zoonotic potential, is also an important environmental contaminant of public health significance (Graves, 1999). Various types of food were deemed to be potentially contaminated with this pathogen. The prevalence was found to differ from place to place, based on hygiene, food content and environmental contamination rate of the specific areas (Graves, 1999). Reports indicated that Listeria spp. including L. monocytogenes is most frequently prevalent in the milk-processing environment including steps, drains and floors (Kells and Gilmour, 2004). Even though pasteurization of raw milk destroys L. monocytogenes, this process does not eliminate later risk of contamination of dairy products (Centre for Disease Prevention and Control, 2013). In addition, a report from Finland indicated that dairy products that are actually made of pasteurized milk might become contaminated by *L. monocytogenes* during subsequent stages of production (Lyytikainen *et al*., 2000).

Infectious Dose: The infectious dose of the organism, as with other pathogens, will be related to the virulence of the particular strain and the host's susceptibility.Controversy exists about the infectious dose. It is clear that most Listeriosis results from ingestion of very high numbers of the organism with 82.9% of cases attributed to ingestion of foods with greater than $1\times10^6$ cfu, it remains unknown if there is a minimum level that can cause illness (FDA, USDA, 2003). However, the ICMSF has reported that "Epidemiologic data indicate that foods involved in listeriosis outbreaks are those in which the organism has multiplied and in general have contained levels well in excess of 100 cfu/g (ICMSF, 2002). Nevertheless because a single microbe has the potential to cause illness (likely in a much debilitated host). The US government has tolerance of negative in 25 g or 0.4cfu/g. In addition the 2003 FDA/USDA in Listeria risk assessment results indicated that cultured milk products, process cheese, hard cheese, ice cream, and other frozen dairy products were found among the very low risk food. A consequence of this risk assessment has been a proposed tolerance in selected ready to eat foods of <100 cfu/g L.mon ocytogenes (FDA, 2008a, b). Essentially L.monocytogenes cannot grow due to low pH (≤ 4.4), lo w water activity (≤0.92), or formulated to prevent L.monocytogenes from growing. According to the reports of Clive and Park (2001) finding *Listeria* spp. prefers to grow at pH 7-8 but they will grow in the range pH 5-10 and may survive and grow in material with a pH as low as 4.4. Recognizing this, frozen foods can prevent Listeria growth and would be acceptable if they contain less than 100 cfu/g of L.monocytogenes if consumed in the frozen state. Hence, the proposed tolerance level may apply to ice cream that is consumed in the frozen state but may not apply to frozen items which must be thawed prior to consumption. Nevertheless non-exempt foods withless than 100 cfu/g made under insanitary conditions would still, likely, be subject to regulatory consequences. If present in raw milk grow and the concentration reached primarily depends on the temperatures and holding times of the milk. Studies of growth of *L. monocytogenes* in raw cows' milk at 4°C indicate that growth of around 1 log10 takes at least five days, and growth of 2 log10 might be expected by 10 days (king *et al*., 2014). This bacterium can survive and multiply at temperature as low as 0°C, but can be easily destroyed under normal cooking temperature. Conditions with pH that ranges from 4.4-9.4 and water activity that equals or is greater than 0.92 may support the growth of L.monocytogenes (WHO/ FAO, 2004) However, a combination of factors (pH, water activity) can also control the growth of it in foods(Codex, 2009).

### 2.6.6 Microbial quality indicators of pasteurized milk

The center for disease control (CDC) in the US, reported that unpasteurized milk is 150 times more likely to cause foodborne illness and results in 13 times more hospitalizations than illnesses involving pasteurized dairy products (USFDA, 2012). Therefore selected microbial tests, that are indicative of the general bacteriological quality, are routinely conducted to evaluate the microbial quality of pasteurized milk. These tests are determination of total bacterial count, coliform count and *E.coli* (Kiiyukia, 2003). Total bacterial count gives quantitative idea about the microbial load present in the sample. Excessively high counts are indicative of poor hygiene and may lead to early spoilage of pasteurized milk that makes it unfit for human consumption.

Coliforms are refered to as indicator microorganisms,while by themselves are not normally causes of serious illness but their presence is used to indicate that other pathogenic organisms of fecal origin may be present (Griffiths, 2000). *E. coli* is a rod-shaped member of the coliform group, unlike the general coliform group, *E. coli* is almost exclusively of fecal origin and its presence is thus an effective confirmation of fecal contamination. Most strains of *E. coli* are harmless, except for serotype O157:H7, which can cause food poisoning in humans (Fernandes, 2009). Coliforms, faecal coliforms, and *E. coli* are destroyed by pasteurization and considered as indicator organisms. These microorganisms can also survive and multiply in a variety of non-intestinal environments, like in the aquatic environment, in soil and on vegetation including the processing plant. Therefore their presence in heat treated milk is indicative of a defective pasteurization process or post pasteurization contamination (Kiiyukia, 2003).

# 3. MATERIALS AND METHODS

## 3.1 Description of Study Site

The study was conducted at Holleta Agricultural Research Centre dairy Farm, Sebeta town, Bishoftu town and in some parts of Addiss Ababa (i.e Jemo one and Tor Hailoch areas).

**Holleta Agriculture Research centers:** is located in the central highland of Oromia special zone surrounding Addis Ababa at a latitude of 38° 30'E, 9° 3'N and 29 km West of Addis Ababa on high way to Ambo. It has an altitude of 2400 m above sea level and receives mean annual rain fall of 1100 mm with bimodal distribution, 70% of which occurs during the main rainy season (June to September) and 30% during the small rainy season (February to April). It has the annual temperature of 11 to 22°C with relative humidity of 50.4%. The soil type in the area is largely vertisol and major crops grown are teff, wheat, barley, oats, potatoes, oil crops and pulses.

**Sebeta towns:** is located at a distance 25km in the south West of Addis Ababa in the Oromia Regional state. Sebeta town has its administrative center. The town is situated at latitude and longitude of 8°55′N 38°37′E and 8.917°N 38.617°E, respectively. The average elevation and temperature of Sebeta town is 2405 m a.s.l.and $22^0$ C, respectively.

**Bishoftu town:** is located at about 45 km South-east of the capital, Addis Ababa and is very close to the other major urban centers like Adama and Mojo. It lies between longitudes 38º51' to 39º04' East and latitudes 8º46' to 8º59' North covering a land area of 1750 $km^2$. Most of the land (90 %) is plain highland. The town is characterized by sub-tropical climate and receives 860 mm rainfall/annum. Mean annual temperature ranges from about 8–28 ºC (Alemayehu *et al.*, 2012). These agro-ecological conditions provide a favorable environment for dairy production. The total population of the district was reached 386,523 (CSA, 2013), which have impact on production systems. The population of Addis Ababa, Adama, Modjo and Bishoftu create a large market for most agricultural commodities. The area is the most developed milk shed of the country, providing most of the dairy products available in the market of Addis Ababa, the largest and most diversified market of Ethiopia (Eshetu, 2008).

### 3.2 Study Population (Study Animals)

The data used in this study were collected from the records of Holeta Agricultural Research Center Dairy farm, private dairy farms and milk vendors of Sebeta and Bishoftu town. In this study, a total of 12 year records of data taken from HARC Holstein-Friesian crosses of lactating cows' data of Holeta Agricultural Research Center (HARC) Dairy farm, which were managed under semi-intensive husbandry systems, was used.The other study populations were dairy farms, milk collectors and milk product seller found in Sebeta and Bishoftu towns.

### 3.3 Research Design

The recorded data analysis were employed to evaluate the effects of season, parity, exotic blood level and lactation stage on milk yield and milk composition of cross bred dairy cows. In addition, cross-sectional survey questioners were used for assessing handling practices of milk and milk products from dairy farmholders and collectors; and a laboratory-based investigation of milk and milk products were conducted to determine the physicochemical, microbial quality and safety of milk samples.

### 3.4 Sampling Techniques

The dairy farms, milk and milk product vendors around study areas were used as sources of milk samples and data through questionnaire survey. Raw milk samples were collected from pooled farm containers of dairy farms and bulk tank containers of milk collectors. However, samples of pasteurized milk, locally produced cheese and yoghurts were collected from super market, open markets and shops in the study areas. All the samples were collected using random sampling method following the procedures bellows.

### 3.5 Dairy Farm Record Data management and Analysis

The 12 years data records of Boran Holstein Friesian crossbred cows were obtained from Holeta Agricultural Research Center dairy farms. Lactation records of cows having their second calving between 2003 and 2014 were used to evaluate the effects of season, parity, genotype (exotic blood level) and lactation stage on milk yield and milk composition. The data was organized and analyzsed according to season, genotype (exotic blood level), lactation stage and parity. On the basis of main prevailing climatic conditions in the study area, the years were classified into two seasons. These includes: the wet season from June to September in which the area gets its major rainfall and the dry season from October to May which receives small and erratic rainfall. Those data records in these two seasons were used to observe the effect of season on milk parameters. To observe the effects of lactation stages on milk parameters, data were categorized into three stages of lactation (Early stage of lactation: 7–105days; Mid-stage of lactation: 106 to 210 days; Late stage of lactation: above 211days) (Gizat, 2004).The data were also divided into 4 categories viz 2, 3,4 and ≥5 parity to observe the effect of parity on milk parameters. Based on their genotype (exotic blood level) cows were also categorized viz 50%, 62.5% and ≥75% exotic blood level to study the effect genotype on milk parameters.

Table 2 Layout of recorded data categories

| Parameter | Boran Holstein Friesian crosses | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Season | Wet | | | | | | | | | | | | Dry | | | | | | | | | | | |
| Exotic blood level | 50% | | | | 62.5% | | | | ≥75% | | | | 50% | | | | 62.5% | | | | ≥75% | | | |
| Parity | 2 | 3 | 4 | 5 | 2 | 3 | 4 | 5 | 2 | 3 | 4 | 5 | 2 | 3 | 4 | 5 | 2 | 3 | 4 | 5 | 2 | 3 | 4 | 5 |
| Lactation stage | | | | | | | | | | | | | | | | | | | | | | | | |
| Early | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| Mid | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| Late | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |

The effects of season, parity, lactation stage and exotic cross level on milk production and compositions were examined using least square mean technique of fitting constants (Harvey, 1990)

### Data statistical analysis

Means and standard errors for the traits studied were estimated using ANOVA procedures of the General Linear Model (GLM) SAS 9.1(2008). The average daily milk yield as affected by season, parity, Friesian cross blood level, stages of lactation and the interactions between these effects were investigated. Differences was considered significant at $P<0.05$. The following model was used to test the effects:

$Y_{ijklm}= \mu + a_i + b_j + c_k + d_l + ab_{ij} + ac_{ik} + bd_{jl} + e_{ijkl}$

In which $Y_{ijklm}$= is the daily milk yield,

$\mu$= the overall mean,

ai=the effect of season (i= dry, wet season)

$b_{j=}$the effect of the dams parity (j= 2, 3, 4 and ≥5),

$c_{k=}$the effect of Friesian blood level (k= 50%, 62.5%, 75%),

$d_l$ =effect of stage of lactation(l= Early, Mid , Late)

$ac_{ik}$, $bd_{jl,}$ the only interactions between the main effects and $e_{ijklm}$ of the random residual effect. The interaction effects were considered for those main effects that were significant.

### 3.6 Questionnaire-based Data Collection and Analysis

The sample size was determined according to Arsham (2005) with a formula of $N=0.25/SE^2$, where SE=3.7%. A total of 183 respondents from dairy farms and milk collecters were selected using simple random sampling techniques. Structured questionnaires containing both open and close ended questions were administered to owners or workers of the different dairy farms and milk collectors, Ayib (cheese) seller in open market and Ergo (yoghurt) seller house in respective districts. Questioner for dairy farms and milk collectors were separately administered ahead milk sample collection where as interview of questentions for Ayib (cheese) and Ergo (yoghurt) were administerd simultaneously during sample collection. The questionnaires were used as a tool to gather information about milk production, handling practices, storage conditions, milking condition and distribution practices. Data related to basic hygiene and handling practices were also collected through visual observation using an observation checklist.

The collected data were coded and tabulated for analysis. The data was analysed using descriptive statistics of SPSS version 16.

### 3.7 Laboratory-based Data Collection

A total of 192 samples of raw, pasteurized milk, cottage cheese and locally fermented yoghurt (Ergo) were collected during July 2017 to June 2018 from Sebeta, Bishoftu and Addis Ababa. All samples were transported in the ice box and subsequently analyzed in the Dairy Laboratory of Holleta Agricultural Research Center.

#### 3.7.1 Milk sample size determination and sample collection

A formula of Kothari (2004) for unknown population (i.e.n = $Z^2SD^2/e^2$) were used to calculate the sample size for this study. Where Z, is the estimated standard variation at 95% confidence interval(CI) which was considered the point of the normal distribution corresponding to the level of significance (Z=1.96). Standard deviation (SD) was estimated at 0.20 or 20% and e, is the estimated error and was considered at 0.05. Therefore, the sample size 'n' was calculated as: n = $[(1.96)^2 \times (0.20)^2]/[(0.05)^2] = 61.4$ approximately n≈61 (for the sake of proportionating the 61 reduced in to 60 samples) of milk and milk products were collected per each areas while 60 samples of milk products were also collected from market sources of study area and additionaly 12 samples of pasteurized milk were collected for comparison from Addis Ababa.

Table 3. Layout of milk and milk products sample collection

| **Sample type** | Sebeta | Bishoftu | Addis Ababa | Overall |
|---|---|---|---|---|
| Farm pooled milk | 30 | 30 | - | 60 |
| Bulk tank milk of collectotrs | 20 | 20 | - | 40 |
| Ayib (cottage Cheese) | 20 | 20 | - | 40 |
| Ergo(locallyfermented Yoghurt | 20 | 20 | - | 40 |
| Pasteurized milk | - | - | 12 | 12 |
| **Total** | 90 | 90 | 12 | **192** |

About 150 ml of fresh raw milk samples from Sebeta and Bishoftu areas were taken using sterile bottles twice at different times aseptically from each dairy farm and milk collectors and kept in an ice box as per the recommendations of (IDF 50 (ISO/DIS 707, 2008). Similarly, 250 ml of pasteurized milk samples were taken twice at different times from similar dairy processing brands. Aseptically collected milk samples in sterile bottles were put in icebox and safely transported to Holleta Agricultural Research Center Dairy Microbiology Laboratory for physicochemical and microbiological analysis. Upon arrival at the laboratory, the samples were asceptically divided in to two. One half of the milk samples for determination of physicochemical properties and the other half were kept for microbiological analysis. Both halves of the samples were kept in sterile containers at a temperature of 4°C until the laboratory analysis.

### 3.7.2 Physicochemical quality test

Physicochemical quality tests included: milk pH, freezing point of milk and specific gravity (milk density) and chemical composition of milk.

#### 3.7.2.1 Specific gravity

The specific gravity of milk samples were measured from individual dairy owner’s cans while they were supplying milk to the milk collection centers. A 50 ml milk sample was put in glass cylinder and the milk was mixed thoroughly and gently to avoid formation of air bubbles. The lactometer was read after it is inserted into the milk and left to float freely until it was at rest. Milk temperature was also read immediately. The milk density was calculated based on the results of the lactometer reading and milk temperature according to the formula described by O’Connor (1994).

Specific gravity= (L/1000) + 1 Where, L: corrected lactometer reading at a given temperature. i.e., for every degree above 60°F, 0.1 degree was added, but for every degree below 60°F, 0.1 degree was subtracted from the lactometer reading.The specific gravity was also taken from lactoscan reading those conforms the lactometer reading was used.

#### 3.7.2.2 Freezing point

Freezing point of the milk was taken from lactoscan reading as soon the samples were taken to Holleta Agriculture Research Center Dairy laboratory.

#### 3.7.2.3 pH

pH of the milk was taken from lactoscan reading as soon the samples were taken to Holleta Agriculture Research Center Dairy laboratory.

#### 3.7.2.4 Milk composition determination and data analysis

Nutritional components of raw and pasteurized milk including percent fat, solids not-fat, lactose, protein, total solids and ash were determined using lactoscan.

The data obtained was tabulated and analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System version 9.1 of SAS (2008). Mean separation were carried out using the Least Significant Difference (LSD) technique when analysis of variance (ANOVA) shows significant differences between means. Differences were considered statistically significant at 5, 1, and/or 0.1% significance level. The following models were used for the milk physicochemical data:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}$$

Where $Y_{ijk}$=individual observation for each samples;

$\mu$=the overall mean;

$\alpha_i$= the $i^{th}$ milk and milk product sources study sites effects (i.e Sebeta, Bishoftu, super market in Addis Ababa)

$\beta_j$=the jth milk and milk product sample type effect ( i.e raw milk, pasteurized milk)

$e_{ijk}$=the error term.

### 3.7.4 Milk microbiological analysis

#### 3.7.4.1 Total aerobic bacterial count (TABC)

To do total aerobic bacterial count, serial dilution was made by adding 1 ml of milk sample into a test tube containing 9 ml of peptone water (Himedia, M028). After thoroughly mixing, the samples homogenates were serially diluted up to $10^{-6}$ (dairy farms and pasteurized milk) and $10^{-7}$(milk collectors). Duplicate samples (1ml) of appropriate serial dilution were pour plated using 15-20 ml molten standard plate count agar (Oxoid, CM0325) and mixed thoroughly. The pour plates were allowed to solidify and then incubated at 32ºC for 48 hours. Colony counts were made using a colony counter according to Robert and Greenwood (2003).

#### 3.7.4.2 Coliform count (CC)

One ml from each of the above sample homogenates, $10^{-3}$ (dairy farms pooled and pasteurized milk) and $10^{-4}$(milk colletor ) serial dilutions were transferred into duplicates of sterile Violet Red Bile Agar (VRBA) (UNI®-CHEM, V37720-2I) plates by the pour plate technique. The resulting inoculated plates were then incubated at $32^0$C for 24 hours. Pink to dark red colonies with bile precipitation around them was counted as coliforms (Robert and Greenwood, 2003).

#### 3.7.4.3 Escherichia coli count (EC)

For the enumeration of Escherichia coli, 25 ml of milk samples were blended in 225 ml of sterile saline solution and serial dilutions were made by adding successively 1 ml of the sample suspension into 9 ml of sterile saline solution. Then 0.1 ml of each of the appropriately diluted milk samples were pour plated on sterile Eosin Methyl Blue Agar (EMB agar) (UNI®-CHEMI, EO3400-2I). The resulting EMB agar plates were incubated at 37°C for 24 hours. Finally, blue black colonies with metallic greenish colonies were counted as E. coli (Roberts and Greenwood, 2003).

#### 3.7.4.4 Yeast and moulds

One ml from each of the above sample homogenates, $10^{-3}$ (dairy farms pooled and pasteurized milk) and $10^{-4}$(milk colletor) serial dilutions were pour plated by Potato Dextrose Agar (Oxide, Pvt. Ltd. MU 096: UK) (i.e before pour plating prepared stock solution of chlortetracycline were added in to the media as antibiotics). The resulting inoculated plates were then incubated at $25^{0}$C for 3 to 5 days. Colonies with a whitish blue green color was counted as yeasts and moulds (Yousef and Carlstrom, 2003).

### 3.7.5 Detection of some pathogenic bacteria

In this method, cell morphology (cell grouping), KOH test, catalase test, motility test, glucose broth confirmatory tests were conducted after cultivating of bacterial colonies in appropriate selective and differential media (Roberts and Greenwood, 2003).

#### 3.7.5.1 Salmonella spp.

A portion of 25 ml of milk were pre-enriched in 225 ml of buffered peptone water (Micro Master, DM 049) at 35°C for 24 hours. Then, 10 ml of pre-enriched sample were incubated in 100 ml Rappaport Vassiliadis soya peptone broth (RVS) at $41.5^{0}$C for 24 hours. Samples from enrichments were streaked on to Xylose Lysine Desoxycholate (XLD) agar (Himedia, M031) media. These selective media was incubated at $37^{0}$C for 24 h and typical colonies with large, glossy black centers or colonies that appeared almost completely black or dark pink to red were considered as *Salmonella spp*. (Roberts and Greenwood 2003).

#### 3.7.5.2 Staphylococcus aureus

Detection of staphylococcus aureus were done according to Godkin and Leslie (1990). Milk samples (25 ml) were diluted in 225 ml of buffered peptone water and mixed in a shaker for 2 minutes. Following this, appropriate dilutions were pour plated by Manitol Salt Agar (Oxoid, CM0085) and incubated at 37°C for 24-48 hours. A yellow zone around the colony indicates mannitol has been fermented. Further, colonies surrounded by yellow zones were transferred to Blood Agar Base (Oxoid) to see β-hemolysis i.e. observing clear zones around the colony

### 3.7.5.3 Listeria monocytogenes

Raw and pasteurized milk samples (25ml) were enriched in 225ml of buffered peptone water (Micro Master, DM 049) at 35°C for 24 hours. Then, 10 ml of pre-enriched sample were incubated in 100 ml of listeria enrichment bronth (half Fraser broth) at 30°C for 24 hours. Samples from enrichments were streaked on to PALCAM agar; incubated the plates at 37°C for a total of 42–48 hours. Colony counts were made using a colony counter according to Robert and Greenwood (2003). After microscopical analysis of the isolates, confirmation were performed using biochemical results (catalase, xylose, mannitol and ramnose fermentation and CAMP test using *Staphylococcus aureus ATCC 25923*) of positive for existence of *L. monocytogenes*. Twenty-five mili grams of milk products (cheese and yoghurt) were also analyzed in the same manner as the raw or pasteurized milk samples.

For all milk and milk products microbiological determination, the number of colony forming units (N) of coliform bacteria per milliliter of sample is calculated using the following formula (Michael and Joseph, 2004).

$$N = \frac{\sum c}{n1 + 0.1\,n2} X d$$

Where,

$\sum c$ = Sum of all colonies on all plates counted

$n_1$ = Number of plates in first dilution counted

$n_2$ = Number of plates in second dilution counted

d = Dilution from which the first counts were obtained

### 3.7.6 Data management and statistical analysis

The microbial count data were first transformed to logarithmic values (log10) before statistical analysis. Then, data on the quality and the transformed microbial count values were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS) version

9.1 of SAS (2008). Mean separation was carried out using the Least Significant Difference (LSD) technique when analysis of variance shows significant differences between means.

The following model was used for the milk microbial count data:

$Y_{ij} = \mu + \alpha_i + \beta_j + e_{ijk}$

Where $Y_{ij}$=individual observation for each test;

$\mu$=the overall mean;

$\alpha_i$= the $i^{th}$ milk and milk product sample study site effects (i.e Sebeta and Bishoftu)

$\beta_j$= the jth milk and milk products sample type effect [raw milk (pooled farm and bulk tank of collectors), pasteurized milk, cheese and yogurt]

$e_{ijk}$=the error terms

# 4. RESULTS

## 4.1 Effects of Season, Parity, Exotic Gene Level and Lactation Stage on Milk Yield and Composition of Holstein Friesian Crosses

### 4.1.1 Effects of season on milk yield and composition of dairy cows

The effects of season on milk yield and milk composition of dairy cows are presented in Table 4. A significant mean difference ($p<0.05$) was observed in milk yield, percent fat and protein due to season. Higher milk yield and percent fat was recorded during dry season while higher protein content was recorded during wet season.Season had no effect on total solid percentage.

### 4.1.2 Effects of genotype on milk yield and composition of dairy cows

The effects of genotype on milk yield and composition of dairy cows are presented in Table 4. Milk yield of 62.5% and ≥75% crossbred cows were significantly higher than that of 50% crossbred cows. Average monthly milk yield of cows of 50%, 62.5% and ≥ 75% crossbred were 215.59, 231.09 and 232.81 liters respectively, which differed significantly ($p<0.05$). Though there was no significant differences in milk components, milk protein of 62.5% crossbred cows was lower in figure than the others.

### 4.1.3 Effects of parity on milk yield and composition of dairy cows

The effects of parity on milk yield and composition of dairy cows are presented in Table 4. Difference in Parity of cow resulted in significantly ($p<0.05$) different yield and protein content of milk. Significantly higher milk yield and protein content was recorded for dam of parity five. An increasing trend was observed in milk yield and protein content as dam parity advances.

### 4.1.4 Effects of stage of lactation on milk yield and composition of dairy cows

The least square means (LSM) for milk yield and composition of the different lactation stages of crossbred dairy cows in Holleta Agricultural Research Center Dairy Farm is indicated in Table 4. Mean monthly milk yield was varied significantly ($p<0.01$) between differe nt lactation stages. As lactation stage advances there was decreasing trend in milk yield and increase in total solid and protein percentages.

Table 4 Mean±SEM monthly milk yield & composition of crossbred dairy cows in Holleta

| Variables | N | Milk yield(l/months) | **Fat%** | **Protein%** | **Total solid%** |
|---|---|---|---|---|---|
| **Season** | | | | | |
| Wet season | 219 | $223.68^{b}$±3.55 | $3.97^{b}$±0.11 | $3.95^{a}$±0.06 | 12.96±0.07 |
| Dry season | 1055 | $229.32^{a}$±1.58 | $4.91^{a}$±0.09 | $3.56^{b}$±0.06 | 13.38±0.13 |
| Significance | | * | * | * | NS |
| **Genotype** | | | | | |
| 50% crosses | 1117 | $215.59^{b}$±1.57 | 4.36±0.09 | 3.90±0.05 | 12.94±0.12 |
| 62.5% crosses | 115 | $231.09^{a}$±4.35 | 4.45±0.12 | 3.44±0.06 | 13.14±0.11 |
| ≥75% crosses | 42 | $232.81^{a}$±4.09 | 4.51±0.15 | 3.91±0.11 | 13.42±0.22 |
| Significance | | * | NS | NS | NS |
| **Parity** | | | | | |
| 2 | 474 | $210.25^{b}$±2.38 | 4.29±0.11 | $3.69^{b}$±0.04 | 13.07±0.05 |
| 3 | 356 | $228.39^{a}$±2.79 | 4.50±0.13 | $3.56^{b}$±0.04 | 13.09±0.07 |
| 4 | 270 | $231.17^{a}$±3.06 | 4.38±0.14 | $3.75^{b}$±0.06 | 12.94±0.08 |
| ≥5 | 174 | $236.20^{a}$±3.26 | 4.59±0.32 | $4.01^{a}$±0.31 | 13.57±0.74 |
| Significance | | * | NS | * | NS |
| **Lactation stage** | | | | | |
| Early | 483 | $240.46^{a}$±2.18 | 4.33±0.11 | $3.65^{b}$±0.04 | $12.90^{b}$±0.06 |
| Mid | 445 | $234.68^{b}$±2.41 | 4.28±0.11 | $3.65^{b}$±0.04 | $12.95^{b}$±0.06 |
| Late | 346 | $204.35^{c}$±2.65 | 4.71±0.19 | $3.95^{a}$±0.16 | $13.65^{a}$±0.38 |
| Significance | | * | NS | * | * |
| **Overall** | 1274 | 218.35±1.45 | 4.62±0.08 | 3.67±0.05 | 13.01±0.11 |

*=p<0.05, NS= Non significant, Lsmean= least square mean, SE=standard error, means in the same column with different superscript letters were significantly different

### 4.1.5 Interaction effect of seasons, genotype, parity and lactation stage on milk yield and composition

The mean squares of milk yield and composition of the interaction of season x breed(S ×G), season×parity(S×P), season× lactation stage(S ×L), genotype ×Parity(G ×P),genotype×lactation( G ×L),parity×lactation stage(P×L) crossbred dairy cows in Holleta agricultural research center of dairy farm is indicated in Table 5. The results of the study showed that interaction of season with genotype and parity with lactation stage significantly influenced milk yield, percentage composition of fat and total solid respectively. Milk yield negatively correlated with fat percent (r=-0.537), protein percent (r=-0.065), total solids percent (r =-0.053). This implies that an increase in milk yield results in a decreasing percentage proportion of Fat, Protein and total solids of milk. Fat percent significantly ($p \leq 0.0001$) correlated with protein percent (r=0.487) and total solids(r=0.531) (Table 6). Protein percent significantly ($p \leq 0.0001$) correlated with Total solids percent (r=0.804).

Table 5. Analysis of variance for interaction of season, genotype ×parity and lactation stage

| | | Mean squares | | | |
|---|---|---|---|---|---|
| **Effect** | **df** | **Milk yield** | **Fat%** | **Protein** | **Total solid** |
| S ×G | 2 | 4915.58* | 25.12* | 0.33 NS | 14.48 NS |
| P ×L | 6 | 3548.6NS | 9.61NS | 4.97 NS | 52.22* |

* indicate statistical significance at the 0.05 probability levels, respectively, DF = Degrees of freedom, NS=non significant

### 4.1.6. Correlation between Physicochemical Properties of Raw Milk

Table 6. Correlation coefficient among milk yield and composition

| | MY | Fat% | Protein% | TS% |
|---|---|---|---|---|
| MY | 1 | | | |
| Fat% | -0.537<br>0.057 | 1 | | |
| Protein% | -0.065(*)<br>0.020 | 0.487(***)<br>0.0001 | 1 | |
| TS% | -0.053<br>0.058 | 0.531(***)<br>0.0001 | 0.804(***)<br>0.0001 | 1 |

MY= MILK YIELD *** Correlation is highly significant at the 0.0001 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed)

### 4. 2 Hygienic Practices and Physicochemical Properties of Raw and Pasteurized Milk

#### 4.2.1 Household Characteristics engaged in milk and its products duties

##### 4.2.1.1 Gender, age and educational status of respondents in Sebeta and Bishoftu areas

Demographic information of respondents is detailed in Table 7. The results showed that most respondents (56.8%) were male with the age range of 20-50 years (81%) and morethan half (53%) had taken formal education of primary and junior school levels of grades 1 to 8. About 95% respondents of the present study either in Sebeta or Bishoftu towns or in over all are litrate only 5% of them are illitrate.

Table 7. Gender, age and educational status of household in Sebeta and Bishoftu towns

| **Variables** | **Sebeta (N) (%)** | **Bishoftu (N) (%** | **Overall (N) (%)** |
|---|---|---|---|
| **Gender** | | | |
| Male | 58(64.1) | 46(50.5) | 104 (56.8) |
| Female | 34(35.9) | 45(49.5) | 79( 43.2) |
| **Age distribution** | | | |
| 20-50 years old | 68(73.9) | 80(87.9) | 148(80.9) |
| 51-65 years old | 11(12) | 8(8.8) | 19(10.4) |
| 66-80 years old | 13(14.1) | 3(3.3) | 16(8.7) |
| **Education level of household** | | | |
| Illiterates | 4(4.3) | 5(5.5) | 9(4.9) |
| Primary and Junior school level(1 to 8) | 48(52.2) | 49(53.8) | 97(53) |
| Secondary school level (9-12) | 37(40.2) | 35(38.5) | 72(39.3) |
| Above secondary school level | 3(3.3) | 2(2.2) | 5(2.7) |

### 4.2.2 Hygienic practices and quality milk production

#### 4.2.2.1 Hygienic practices in barns and at milk production in Sebeta and Bishoftu areas

Barn and udder cleaning practice in Sebeta and Bishoftu dairy farms are shown in Table 8. About 92.3% of the respondents in the study areas clean the barn twice per day, while 7.7% clean once a day. Farms from Bishoftu showed better cleaning practices (95.5%) than farms in Sebeta where only (89.1%) respondantes practiced their barns twice per day. Cleaning the udder of cows before milking is important since it could have direct contact with the ground, urine, dung and feed refusals while resting. However, 61.8% of respondents washed the udder before milking.

Table 8. Barn types, fequencey of barn cleaning and udder washing practices in Sebeta and Bishoftu towns

| Variables | Sebeta N (%) | Bishoftu N(%) | Overall N (%) |
|---|---|---|---|
| **Barn Types or its flooring** | | | |
| Separate housing | | | |
| • Soil | 12(13) | 12(13.2) | 25(13.6) |
| • concrete | 80(87) | 79(86.8) | 159(86.4) |
| **Frequency of cleaning barn** | | | |
| • Twice /day | 82(89.1) | 87(95.6) | 169(92.3) |
| • Once /day | 10(10.9) | 4(4.4) | 14(7.7) |
| **Udder washing** | | | |
| Clean before milking | 42(45.7) | 71(78) | 113(61.8) |
| Clean after milking | 37(40.2) | 20(22) | 57(31.1) |
| Clean before and after milking | 13(14.1) | 0(0) | 13(7.1) |

#### 4.2.2.2 Milking and milk handling practices in Sebeta and Bishoftu towns

The survey results showed that cleaning of milk handling equipment is a common practice among the respondents of the study areas. Accordingly, all the respondents(100%) of the two study areas wash milk equipments twice using wam water (45.4%), detergent plus either warm or cold wateralternatively (41.5%) and 13.1% of them used only cold water (Table 9). About 96.2 and 3.8 percent of the respondents used plastic and stainless steel equipment respectively. Most (72.7%)of respondents used table to dry and store the cleaned containers. Majoriy of milk suppliers (74.3%) were located in a distance between 500 and 1000 meters where as 24.6 percent were located at a distance below 0.5km. Plastic containers used by respondents in this study were blamed for their adhesive properties which cannot be cleaned easily.

Table 9 .Milking and milk handling practices in Sebeta and Bishoftu towns

| Variables | Sebeta N(%) | Bishoftu N(%) | Overall N(%) |
|---|---|---|---|
| **Frequency of milking /day (%)** | | | |
| Twice | 92(100) | 91(100) | 183(100%) |
| Once | 0 | 0 | 0 |
| **Milker** | | | |
| House hold head/spouse | 76(82.6) | 65(71.4) | 141(77.1%) |
| Son/daughter | 1(1.1) | 4(4.4) | 5(2.7) |
| Employee | 15(16.3) | 22(24.2) | 37 (20.2) |
| **Milk Utensils** | | | |
| Plastic | 88(95.6) | 88(96.7) | 176(96.2%) |
| Aluminum | 4(4.4) | 3(3.3) | 7(3.8%) |
| **Frequency of washing utensils** | | | |
| Once | 0 | 0 | 0 |
| Twice | 92(100) | 91(100) | 183(100) |
| **Cleaning the container** | | | |
| After each usage cold water alone | 12(13) | 12(13.2) | 24(13.1) |
| After each usage hot water alone | 61(66.3) | 22(24.2) | 83(45.4) |
| Alternatively Cold and Hotwater + soap | 19(20.7) | 57(62.7) | 76(41.5) |
| **Clean container storing** | | | |
| On table | 67(72.8) | 66(72.5) | 133(72.7) |
| Hanging | 23(25) | 21(23.1) | 44(24) |
| On ground | 2(2.2) | 4(4.4) | 6(3.3) |
| **Distance /time spent on delivery** | | | |
| 50-500 m | 17(18.5) | 28(30.8) | 45(24.6) |
| 500-1000 m | 75(81.5) | 61(67) | 136(74.3) |
| >1000m | 0(0) | 2(2.2) | 2(1.1) |

### 4.2.3 Physicochemical properties of milk

#### 4.2.3.1 Hydrogen ion concentration (pH)

The mean pH of milk samples from pooled farm containers of producers, bulk tank containers of collectors and pasteurized milk from Sebeta, Bishoftu area and Addis Ababa is shown in Table 10.The milk collected from Sebeta area had an average milk pH of 6.28, whereas that of Bishoftu area was 6.49. The pH of farm pooled milk samples from Bishoftu area was significantly higher than the pH other samples. The pH of milk samples from Bishoftu area was almost similar to the required standard but that of Sebeta was slightly lower.

#### 4.2.3.2 Density/Specific gravity

The mean specific gravityof milk samples from pooled farm containers of producer, bulk tank containers of collectors and pasteurized milk from Sebeta, Bishoftu area and Addis Ababa is shown in Table 10.There was a significant difference ($P<0.001$) in specific gravity among the study areas and the value of specific gravity in general falls within the ranges of Ethiopia standard (ES) value. However, pasteurized milk had significantly lower specific gravity than the raw milk samples and lower than that of the Ethiopian standard.

#### 4.2.3.3 Freezing point

The mean freezing point of milk samples from pooled farm containers of producer, bulk tank containers of collectors and pasteurized milk from Sebeta, Bishoftu area and Addis Ababa is shown in Table 10. In this study, the freezing point of milk from Sebeta area ($-0.619^{\circ}C$) was significantly ($P<0.01$) lower than Bishoftu area ($-0.486^{\circ}C$). The freezing point of pasteurized milk was not in the range of Ethiopian standard.

Table10. Mean raw and pasteurized milk preliminary quality test of Sebeta and Bishoftu areas

| Study sites | N | Sp.gravity | Freezing pt | pH |
|---|---|---|---|---|
| **Sebeta** | | | | |
| Farm pooled | 30 | $1.029^{b}\pm0.000$ | $-0.528^{b}\pm0.008$ | $6.28^{b}\pm0.07$ |
| Bulk tank | 20 | $1.033^{a}\pm0.002$ | $-0.619^{a}\pm0.024$ | $6.28^{b}\pm0.04$ |
| **Bishoftu** | | | | |
| Farm pooled | 30 | $1.028^{bc}\pm0.000$ | $-0.525^{b}\pm0.005$ | $6.56^{a}\pm0.05$ |
| Bulk tank | 20 | $1.026^{c}\pm0.001$ | $-0.486^{b}\pm0.012$ | $6.31^{b}\pm0.02$ |
| **Pasteurized** | 12 | $1.023^{d}\pm0.001$ | $-0.422^{c}\pm0.023$ | $6.01^{c}\pm0.41$ |
| Significance | | *** | *** | *** |

***=p<0.001, SE=standard error, means in same column with different superscript letters were significantly different.

#### 4.2.4 Milk composition of Sebeta and Bishoftu areas.

##### 4.2.4.1 Lactose content of milk samples

The overall mean and standard error of lactose content of milk sampled from Sebeta and Bishoftu area is shown in Table 11. Lactose content of milk sampled from Sebeta area was significantly higher than the sample from Bishiftu area and that of pasteurized milk sample.

##### 4.2.4.2 Protein content of milk samples

The overall mean and standard error protein content of milk sampled from Sebeta and Bishoftu area is shown in Table 11. Protien content of milk sampled from Sebeta area (3.28±0.08) was higher than that of Bishoftu area (2.90±0.04). There was significant difference in protein content between the two study areas and among milk samples (P<0.01) with the higher content recorded from Sebeta area. Milk sampled from bulk tank of Sebeta area had highest protein content while pasteurized milk samples had lowest protein content.

#### 4.2.4.3 Fat content of milk samples

The overall mean and standard error means of fat content of milk sampled from Sebeta and Bishoftu areas are shown in Table 11. Fat content of milk sampled from Sebeta area was higher than that of Bishoftu area**.**The overall value of fat(3.68%) sampled from Sebeta area was higher than that of Ethiopian standard values (3.5%). But for Bishoftu area, the milk fat content (3.59%) was almost similar with that of Ethiopian standard values (3.5%)**.**

#### 4.2.4.4 Solids not fat content of milk samples

The overall mean and standard error of means of solid not fat content of milk sampled from sebeta and Bishoftu areas are shown in Table 11. Solid not fat content of milk sampled from Sebeta area was higher than that of Bishoftu areas .The overall values of this study for SNF% sampled from Sebeta area higher than that of Bishoftu area**.**

#### 4.2.4.5 Total solids content of milk samples

The overall mean and standard error of percent total solid of milk samples from Sebeta and Bishoftu area are shown in Table 11. Total solid percent of milk sampled from Sebeta area was higher than that of Bishoftu area**.** The overall total solids% of milk samples collected from Sebeta areas was less than the value (12.8%) set by Ethiopian standard.

#### 4.2.4.6 Total ash content of milk

The overall mean and standard error of means of percent ash of milk sampled from Sebeta and Bishoftu areas are shown in Table 11.Total ash percentage of milk sampled from Sebeta area was higher than that of Bishoftu area**.**

### 4.2.5 Physicochemical composition of pasteurized milk

The overall mean chemical composition of pasteurized milk from Addis Ababa supermarkets and shops are shown in Table 11. The percent lactose, protein, solid not fat (SNF), total solid and ash contents of pasteurized milk were significantly lower than that of cows milk sampled from Bishoftu and Sebeta areas.

Table 11. Mean±SEM raw and pasteurized milk content of Sebeta and Bishoftu areas

| Study sites | N | Lactose | Protein | Fat | SNF | TS | Ash |
|---|---|---|---|---|---|---|---|
| **Sebeta** | | | | | | | |
| Farm pooled | 30 | $4.57^{b}$±0.06 | $3.05^{b}$±0.04 | $3.50^{ab}$±0.23 | $8.31^{b}$±0.11 | $11.81^{b}$±0.26 | $0.69^{b}$±0.01 |
| Bulk tank | 20 | $5.25^{a}$±0.18 | $3.50^{a}$±0.12 | $3.86^{a}$±0.27 | $9.54^{a}$±0.33 | $13.40^{a}$±0.56 | $0.79^{a}$±0.03 |
| **Overall Seb.** | **50** | **4.91±0.12** | **3.28±0.08** | **3.68±0.25** | **8.93±0.22** | **12.61±0.41** | **0.74±0.02** |
| **Bishoftu** | | | | | | | |
| Farm pooled | 30 | $4.51^{bc}$±0.03 | $3.00^{bc}$±0.02 | $3.49^{ab}$±0.11 | $8.21^{bc}$±0.05 | $11.70^{b}$±0.13 | $0.68^{bc}$±0.00 |
| Bulk tank | 20 | $4.21^{c}$±0.09 | $2.80^{c}$±0.06 | $3.69^{ab}$±0.15 | $7.65^{c}$±0.16 | $11.34^{b}$±0.27 | $0.63^{c}$±0.01 |
| **Overall Bish.** | **50** | **4.36±0.06** | **2.90±0.04** | **3.59±0.13** | **7.93±0.11** | **11.52±0.20** | **0.66±0.01** |
| Pasteurized | 12 | $3.72^{d}$±0.19 | $2.48^{d}$±0.12 | $3.01^{b}$±0.15 | $6.77^{d}$±0.33 | $9.78^{c}$±0.47 | $0.56^{d}$±0.03 |
| Significance | | *** | *** | *** | *** | *** | *** |

***=p<0.001, NS= Non significant, SE=standard error, Means in the same column with different superscript letters are significantly different, Seb.= Sebeta, Bish.=Bishoftu

### 4.2.6. Correlations among the physicochemical parameters of raw milk

The correlation between physico-chemical properties of raw milk in the study areas is shown in Table 12. Protein content was strongly correlated with ash, SNF, lactose, specific gravity, total solid and freezing point of milk. The correlation appears positive and confirms the fact that the higher protein value is associated to the higher other components. Fat content of milk was weakly correlated with protein, ash, SNF, lactose, specific gravity, total solid and freezing point of milk. Specific gravity of milk was strongly correlated with protein, ash, SNF, lactose, total solid and freezing point of milk.

Table 12. Correlation between physico-chemical properties of raw milk in the study areas

| Variable | Tot. solid | Protein | Fat | Ash | SNF | Lactose | pH | Sp. grav. | Freez. pt. |
|---|---|---|---|---|---|---|---|---|---|
| Tot. solid | 1 | | | | | | | | |
| Protein | 0.830*** | 1 | | | | | | | |
| Fat | 0.820*** | 0.357** | 1 | | | | | | |
| Ash | 0.830*** | 0.996*** | 0.367** | 1 | | | | | |
| SNF | 0.830*** | 0.9980*** | 0.359** | 0.994*** | 1 | | | | |
| Lactose | 0.830*** | 0.999*** | 0.364** | 0.996*** | 0.998*** | 1 | | | |
| pH | 0.086$^{NS}$ | 0.012 $^{NS}$ | 0.123 $^{NS}$ | 0.083*** | 0.021 $^{NS}$ | 0.014 $^{NS}$ | 1 | | |
| Sp. grav. | 0.834*** | 0.999*** | 0.364** | 0.996*** | 0.998*** | 0.999*** | 0.014$^{NS}$ | 1 | |
| Frezg. pt. | 0.864*** | 0.988*** | 0.423*** | 0.984*** | 0.989*** | 0.999*** | 0.033$^{NS}$ | 0.988*** | 1 |

** Correlation is significant at the 0.001 level (2-tailed).
***.Correlation is significant at the 0.0001 level (2-tailed).
NS = Non significant difference

### 4.3. Microbial Quality and Food Born Pathogens in Milk and Milk Products of Sebeta and Bishoftu Towns

#### 4.3.1 Aerobic mesophilic bacterial count.

The least square mean of aerobic mesophilic bacterial count (AMBC) in samples from containers of pooled farm and collectors bulk tank milk, cottage cheese (ayib), local yoghurt (Irgo) of Sebeta and Bishoftu towns and pasteurized milk samples are presented in Table 13.

There was no significant difference in TABC of raw milk due to difference in location of sampling.But there was significant difference in TABC within study location due to raw milk sampled from farm pooled of producer and bulk tank of collectors of Sebeta town. There was also no significant difference in TABC due to difference in study site of sampling either in ayib (local cheese) and Irgo (local yoghurt) sampled from Sebeta and Bishoftu towns.Higher total aerobic bacterial count (7.33±0.09log10cfu/ml) was found on milk samples collected from bulk tank of collectors. Pasteurized milk of this study showed the least total aerobic bacterial counts (5.21log10cfu/ml).

### 4.3.2 Total coliform count

The least square mean of total coliform count (TCC) in samples from containers of pooled farm and collector bulk tank milk, cottage cheese (ayib), local yoghurt (Irgo) of Sebeta and Bishoftu towns and pasteurized milk samples are presented in Table 13.

Total coliform counts was significant higher in Bishoftu's raw milk samples than Sebeta towns. There was no significant variation in TCC with in Sebeta raw milk but higher in TCC in milk samples of bulk tank milk of collectors of Bishoftu than its respective farm pooled milk of producer of Bishoftu town. TCC in yoghurts of Sebeta town is singnificantly higher than Bishoftu town.Cheese sample was not showed significant variation in TCC. Pasteurized milk samples of the present study showed lowest total coliform counts.

### 4.3.3 Escherchia coli

The least square mean of Escherchia coli count (E.coli count) in samples from containers of pooled farm and collector bulk tank milk, cottage cheese (ayib), local yoghurt (Irgo) of Sebeta and Bishoftu towns and pasteurized milk samples are presented in Table 13.

The analysis of variance showed that there was significant difference in E.Coli counts within and between study location due to raw milk sampled from farm pooled of producer and bulk tank of collectors of either Sebeta or Bishoftu towns. There was also significant variation in E.coli count due to yoghurt samples from different study sites.

Relatively lowest E.coli count was identified in pasteurized and yogurt samples. Overall prevalence of E.coli in the present study was 80% in farm pooled samples, 90% of bulk tank milk samples, 90% of yoghurt samples, 75% of cheese samples and 33.34% of pasteurized milk samples (Table 14).

#### 4.3.4 Yeast and mould count

The least square means of yeast and mould count of milk and milk products sampled from Sebeta and Bishoftu areas are shown in Table 13. Significant variations in yeast and mold count were observed due to difference of raw milk sample collection sites and milk and milk product sample types. There was no significant difference in yeast and mould count in cheese and yoghurt samples due to difference in study site. Higher yeast and mold count were found on raw milk samples collected from Bishoftu.The lowest yeast and mould counts were identified in pasteurized milk samples.

#### 4.3.5 Staphylococuss aures

The mean Staphylococcus aures count of milk and milk products sampled from Sebeta and Bishoftu area is shown in Table 13.There was a highly significant difference in S. aures count due to different study sites in the raw milk and cheeses sample types. Higher S.aures count were identified in raw milk and cheese samples from Bishoftu town and it was highest in farm pooled milk samples.S.aures was identified in 95% farm pooled samples, 90% of bulk tank milk samples, 77.5% of yoghurt samples , 82.5% of cottage cheese samples and 50% of pasteurized milk (Table 14). This study finding showed higher count values in raw milk samples (93%) than othr products.

#### 4.3.6 Listeria monocytogenes

The least square mean of Listeria monocytogenes count of milk and milk product sampled from Sebeta and Bishoftu area is shown in Table 13. The results showed that, there were significant differences due to sites of raw milk sampling but sites of sampling was not showed significant differences on L. monocytogenes counts in samples of cheese and yoghurt. Lower counts of L. Monocytogenes was identified on cottage cheese and yoghurt samples whereas higher counts were found from samples of farm pooled, bulk tank and pasteurized milk. In this study L. Monocytogenes was identified in 70% farm pooled samples, 85% of bulk tank milk samples, 52.5% of yoghurt samples, 65% of cottage cheese samples and 75% of pasteurized milk samples (Table 14)

#### 4.3.7 Salmonella

The overall prevalence of salmonella in raw milk (farm pooled and bulk tank) of this study was 9% (9 out of 100) whereas, 6.7% for farm pooled milk (each 2sample of thirty samples of Sebeta and Bishoftu), 12.5% prevalence of bulk tank milk (3 out of 20samples of Sebeta and 2 out of 20 samples of Bishoftu area),10% prevalence of it in yoghurt( 4 out of 20 samples of Sebeta and 0 out 20 samples of Bishoftu), 22.5% prevalence of it in cheese (3 out of 20 samples of Sebeta and 6 out of 20 samples of Bishoftu)(Table 14). Salmonella was not detected in pasteurized milk of this study. Higher prevalence of Salmonella was found in cheeses and bulk tank milk.

#### 4.3.8 Pasteurized milk

The Least Square mean microbial count of different brand pasteurized milk samples obtained from supermarket, shops and kiosk of some parts of Addis Ababa is shown in Table 13. Microbial loads of pasteurized milk were significantly lower than that of the raw milk and other milk products samples.The results showed that pasteurized milk had the counts of total aerobic b acterial of (5.21±0.14), coliform (2.74±0.54), Escheria coli (1.07±0.21), yeast and mould (3.64±0 .19), S.aures (1.91±0.46) but L.monocytogenes (4.60±0.65) log10cfu/ml.

Table13.Mean microbial count (log10cfu/ml) of milk and its products of Sebeta & Bishoftu town

| Parameters | N | TABC | T. COL. | YMC | S. aurus | E. coli | L. Monnoc. |
|---|---|---|---|---|---|---|---|
| **Sebeta** | | | | | | | |
| Farm pooled(FP) | 30 | 6.80$^{b}$±0.12 | 3.75 ±0.11 | 4.83 $^{a}$±0.11 | 3.97 ±0.08 | 3.40$^{b}$±0.09 | 4.31±0.25 |
| Bulk tank(BT) | 20 | 7.33 $^{a}$±0.09 | 3.68 ±0.26 | 4.42$^{b}$±0.08 | 3.73 ±0.11 | 3.76$^{a}$±0.09 | 4.63±0.09 |
| **Significance** | | * | **NS** | * | **NS** | * | **NS** |
| **Sebeta raw (FP+BT) milk** | **50** | **7.01$^{a}$±0.09** | **3.72$^{b}$±0.12** | **4.67$^{b}$±0.08** | **3.88$^{b}$±0.08** | **3.54$^{a}$±0.07** | **4.44$^{b}$±0.15** |
| **Bishoftu** | | | | | | | |
| Farm pooled(FP) | 30 | 7.31 ±0.06 | 3.80$^{b}$±0.36 | 5.51±0.07 | 5.39 $^{a}$±0.05 | 2.20$^{b}$±0.30 | 4.20$^{b}$±0.22 |
| Bulk tank(BT) | 20 | 7.18 ±0.25 | 5.50 $^{a}$±0.13 | 5.42 ±0.04 | 4.88$^{b}$±0.14 | 4.10 $^{a}$±0.42 | 5.01 $^{a}$±0.19 |
| Significance | | **NS** | * | NS | *** | *** | * |
| **Bishoftu raw milk** | **50** | **7.26$^{a}$±0.11** | **4.48$^{a}$±0.25** | **5.48$^{a}$±0.05** | **5.19 $^{a}$ ±0.07** | **2.96$^{b}$±0.28** | **4.52$^{a}$±0.16** |
| **Ayib (local Cheese)** | | | | | | | |
| Sebeta Cheese | 20 | 6.93 ±0.23 | 3.70±0.28 | 4.93 ±0.09 | 2.83$^{b}$±0.33 | 3.11±0.16 | 1.61 ±0.47 |
| Bishoftu Cheese | 20 | 6.66 ±0.25 | 4.14 ±0.40 | 5.03 ±0.17 | 3.98$^{a}$±0.22 | 2.63 ±0.28 | 2.36 ±0.60 |
| **Significance** | | NS | NS | NS | ** | NS | NS |
| **Irgo(local yoghurt)** | | | | | | | |
| Sebeta Yoghurt | 20 | 6.85±0.25 | 4.57 $^{a}$±0.35 | 4.74 ±0.19 | 3.78 ±0.14 | 1.72$^{b}$±0.25 | 2.74 ±0.52 |
| Bishoftu Yoghurt | 20 | 6.43 ±0.35 | 3.35 $^{b}$ ±0.23 | 4.44 ±0.16 | 3.50 ±0.38 | 2.93 $^{a}$±0.20 | 2.75 ±0.39 |
| **Significance** | | NS | * | NS | NS | *** | NS |
| Pasteurized milk | 12 | 5.21±0.14 | 2.74±0.54 | 3.64±0.19 | 1.91±0.46 | 1.07±0.21 | 4.60±0.65 |

**=P<0.01, ***=P<0.001, NS= Non significant, Lsmean= least square mean, SE=standard error,[a,b,c,d] Means in the same column with different subscript letters were significantly different, YMC =yeast and mould counts

Table 14 Number (Prevalence) of bacteria in milk and milk products of study areas

| Sample site and type | No of samples | Stap. aures | E.coli | L. Monocytogen. | Salmonella |
|---|---|---|---|---|---|
| **Sebeta** | | | | | |
| Farm pooled | 30 | 28(93.3) | 25(83.3) | 24(80) | 2(6.7) |
| Bulk tank | 20 | 17(85) | 20(100) | 20(100) | 3(15) |
| **Sebeta raw milk** | 50(=30+20) | **45(90)** | **45(90)** | **44(88)** | **5(10)** |
| Cheese | 20 | 15(75) | 17(85) | 8(40) | 3(15) |
| youghurt | 20 | 15(75) | 20(100) | 14(70) | 4(20) |
| **Bishoftu** | | | | | |
| Farm pooled | 30 | 29(96.7) | 23(76.7) | 26(86.7) | 2(6.7) |
| Bulk tank | 20 | 19(95) | 16(80) | 20(100) | 2(10) |
| **Bishoftu raw milk** | **50**(=30+20) | **48(96)** | **39(78)** | **46(92)** | **4(80** |
| cheese | 20 | 18(90) | 13(65) | 20(100) | 6(30) |
| youghurt | 20 | 16(80) | 16(80) | 12(40) | 0 |
| **Pasteurized** | 12 | 6(50) | 4(33.3) | 9(75) | 0 |
| Total | 192 | | | | |

# 5. DISCUSSIONS

## 5.1 Effects of Season, Parity, Exotic Gene Level and Lactation Stage on Milk Yield and Compositions of Holstein Friesian Crosses

### 5.1.1 Effects of season on milk yield and composition of dairy cows

Milk yield and composition of dairy cows can be affected by season of milking. A significant variation was observed in milk yield, percent fat and protein due to season of milking. Higher yield and percent fat was recorded during dry season but vice-versa for protein, whereas percentage composition of total solid of milk was not affected due to seasons.

The present results were in agreement with reported values of 4.53% fat of milk (Sharma *et al.*, 2002); finding of higher fat content in dry season than wet season (Cheruiyot *et al.*, 2018); higher protein(casein) contents in the wet season than in the dry season(Cheruiyot *et al.,*2018; Mesfin and Getachew, 2007).Studies justified that milk protein level in the hot months was less than wet season due to the decrease in the casein (Bernabucci *et al.*, 2002) and season affects milk protein in that hot and humid months depress protein content (Melaku and Gurmessa, 2012).Cheruiyot *et al. (*2018) also reported similar findings,where TS contents were not affected by the month of sampling.

Unlike this finding different researcher reported that season had no effect on milk yield (Baset *et al.*, 2016; Angel *et al.*, 2013; Bilgic and Alic, 2005; Pelister *et al.*, 2000), TS content varied among seasons being highest in winter (Sharma *et al.*, 2002), higher milk fat and total solids content of Boran-Friesian crossbred dairy cows in July to September but the lowest total solids content in January to March reported by Mesfin and Getachew (2007). Melaku and Gurmessa (2012) reported that season affects milk fat where hot and humid months depress fat content of milk.

The CP and rumen degradable protein (RDP) intake of cows were higher in dry season compared to that of wet season due to shortage of quality and quantity of roughage feeds hence milk proteins and fatty acids are influenced by seasonal changes (Heck *et al.* 2009). The high protein content during wet season was probably either due to good availability of green feed or the cool temperature favors its metabolic synthesis.

### 5.1.2 Effects of genotype on milk yield and composition of dairy cows

Milk yield and composition of dairy cows can be affected by exotic blood level in crossbred cows. An increase in exotic blood level results in an increase in milk yield. Similar findings were reported by different researchers where Galukande *et al.* (2013) reported that in the highland climatic zone, the mean lactation milk yield for cows with exotic inheritance of 50 percent B. taurus genes was 2.6 times and that of 75 percent B. taurus genes was 2.7 times higher than that of the indigenous cows respectively. Nantapo (2012) also affirmed that milk yield and fat content of milk differ in different genotypes. Further more, finding by Kennedy *et al.*(2003) and Turki *et al.*(2012) agreed with the current results where high merit cows had the highest yield of milk and the low merit cows had lowest yield of milk. In the present study milk yield of 62.5% was not significantly differed from that of 75% exotic blood levels .The finding of Addisu (2013) and Mohamed *et al.(*2001) revealed that as blood level increased, reduction in yield performance was observed. The same finding justified that the reduction in performance could be due to reduction in epistatic effect and effect of recombination as there have a negative effect on productivity of cross breeds.

Similar findings were also reported by Cheruiyot *et al.* (2018) where there was no significant difference on milk composition traits due to difference in proportion of exotic genes in crossbred dairy cows.

Contrary to this study, Aynalem *et al.* (2008) and Islam *et al.* (2014) reported that increasing the proportion of exotic genes in a cow leads to decreased milk component levels,whereas Cheruiyot *et al.*(2018) reported an inceasing percent of fat and protein up to>84% exotic gene level but reduction in all component beyond this blood level.

In overall, the results of this study didn't show variation in chemical quality standard due to effect of crosses gene lvel, therefore, percent of fat, protein and total solid fulfills Ethiopian, Europian and FAO milk quality standard requirements. This finding is in agreement with Desyibelew *et al*. (2019) who concluded that the Zebu × HF crossbred animal was the good performer regarding the milk composition and quality.

### 5.1.3 Effects of parity on milk yield and composition of dairy cows

The difference in parity of cow significantly affected milk yield and protein content of milk where an increasing trend in milk yield and protein contents were observed as dam parity advances. Similar results were reported by different researchers where there is an increase in milk yield with the increasing age/ parity (Zewdu *et al*., 2013; Ángel *et al*., 2013; Bath *et al*., 1985 and Afzal *et al*., 2007). The report of Niraj *et al*.(2017) yield results of 242.9±2.6 liter/momths and 2503.6 ± 76.8 litres/lactation conforms this study. Lee and Kim (2006) also reported finding in agreement with this results that there is an increase in milk yield towards 5$^{th}$ parity and decline thereafter, fat content was not significantly varying with parity (Jemila and Achenef, 2012), Shuiep *et al*.(2016) also revealed that no significant influences for parity order on milk fat content although it tends to decrease in the later parities.

Unlike this, findings by researchers revealed that milk yield and protein contents of the milk did not show significant variation in different parity groups (Jemila and Achenef, 2012; Shuiep *et al*., 2016)

An increasing trend in milk yield and protein content as dam parity advances in present study agrees with the findings of Bath *et al.(*1985) who reported that an increase in milk yield with the increasing age was partially attributed to higher body weight and increased development of the udder during recurring pregnancies which results in larger mass of digestive system and mammary glands for synthesis of milk. In overall, the results of study were with in the quality standards of parity effect, hence percent fat, protein and total solid fulfills Ethiopian, Europian and FAO milk quality standard requirements.

### 5.1.4 Effects of stage of lactation on milk yield and composition of dairy cows

The milk yield and composition of crossbred dairy cow were significantly affected by different stages of lactation. As lactation stage advances there was decreasing in milk yield and increasing in percent composition of total solid and protein.

Many findings were in agreement with this in that highest milk yield was observed in the early lactation stage then it decreased gradually until the end of lactation where the lowest yield was recorded (Baset *et al.*,2016; Mohamoud *et al.,*2014: Carlos , 2013 and Agnihotri and Rajkumar, 2007). Similarly Anila and Muhammad (2009) reported higher yield in early lactation but differently vice-versa for mid and late lactation in milk yield. In the present study fat percentage composition was not significantly affected by advancing lactation stages though there is an increasing trend in values. Jemila and Achenef (2012) reported high fat content in late stage of lactation which agrees with this finding.

Unlike this finding Jemila and Achenef (2012) reported that milk yield did not show significant variation in different stage of lactation, higher fat content in early than mid lactation.

The obtained results support conclusion of diffent findings in that stage of lactation is one of the major factors influencing milk yield and composition in dairy cows (Shuiep *et al.*, 2016; Ibeawuchi and Dangut, 1996)

### 5.1.5 Interactions and correlation between seasons, genotype, parity and lactation stage on milk yield and composition

Result of study showed that interaction of season with genotype and parity with lactation stage significantly influenced milk yield, percentage composition of fat and total solid respectively. Milk yield negatively correlated with fat% ($r = -0.537$), protein% ($r=-0.065$), total solid($r = -0.053$). Fat% significantly ($p \leq 0.0001$) correlated with protein% ($r=0.487$) and total solid ($r=0.531$). This implies that an increase in milk yield results in a decreasing percentage proportion of Fat, protein and total solid of milk. Protein% significantly ($p \leq 0.0001$) correlated with Total solid ($r=0.804$). This finding agrees with the finding of Baset *et al.*(2016) reported where milk yield was negatively correlated with the percentage of fat, protein, lactose and SNF that milk composition strongly correlated with each other. This finding is supported by finding of Anila and Muhammad (2009) who reported that milk protein and fat percentages are inversely related to milk yield.

## 5.2 Hygienic Practices and Physicochemical Quality of Raw and Pasteurized Milk of Sebeta and Bishoftu areas

### 5.2. 1 Household characteristics

#### 5.2.1.1 Gender, age and educational status of respondents of Sebeta and Bishoftu areas

In this study in overall, among interviewed respondents of dairy keepers 56.8% of them were male with the age range of 20-50 years and majorly (53%) had taken formal education of primary and junior school level (Table 7). This finding result was less than what has been reported for Mekelle Negussie (2006), of 73% for male-headed, for Addis Abebe (75.9%), (Yoseph *et al*., 2003), Awassa (76.7%) (Ike, 2002) and Bahir Dar and Gonder (77%) (Yitaye, 2008). This indicates that there is an increase in women involvement in dairy sector than earlier time.The high percent of female-headed households (43.2%) in the present study might be due to better access for market to sell milk and encouraging opportunity of credit services from different micro finance institutes. About 95% of the dairy farmers in the study are litrate; this is good opportunity in the study area for adoption of new technology in the development of dairy sector.

### 5.2.2 Hygienic practices for milk production

#### 5.2.2.1 Hygienic practices in barns and at milk production in Sebeta and Bishoftu areas

Maintaining the sanitary condition of barn is important for the production of good quality milk. That is clean, dry and comfortable bedding condition minimizes the growth of pathogenic microorganisms.The practice of cleaning the barn in the study area was better in that the majority (92.3%) clean the barn twice a day which is better than the practice reported by Bereda *et al.*

(2012) whereh about 47% of the producer clean their barn sthree times a week. Almost similar results were reported by Yilma (2012) that about 87% producers in from Addis Ababa cleaned their barns on daily bases and more than 90% Abate *et al*. (2015) report from Jigjiga City of Somali region cleaned barns once a day.Unlike this finding Debala (2015) reported that, 56.7% respondents in Yabello district of Borena zone clean the barn once a week, while 38.9% clean more than once a week and 4.4% of them clean once a month. Similar study from Gondar reported that about 88.3% of respondents clean the barn more than twice a week, but 11.7% of respondents clean the barn twice per week (Tegegne and Tesfaye, 2017). Proper and clean housing environment is a prerequisite to produce milk and milk products of acceptable quality (Tassew, 2007).

#### 5.2.2.2 Milking and milk handling practices in Sebeta and Bishoftu towns

Cleaning of milk handling equipment is common among most of the interviewees. The equipment used for milking, transportation and storage determine the quality of milk and milk products. Milk containers types, especially those used for transportations of milk to the delivery point greatly determine the qualities of milk. Producers need to pay attention for the type as well as cleanliness of milk equipment (Debala, 2015). About 96.2% and 3.8% of respondents used plastic and stainless steel equipment respectively. All the respondent milks their cow twice a day and 45.4% of respondents wash the milk utensil after each usage using hot water alone whereas 41.5% uses alternatively cold and hot water and detergents, the remaining respondents use cold water alone. Most (72.7%) of respondent used table to dry the cleaned container. Earlier study in Bishoftu by Desalegn (2017) reported similar finding that majority of the interviewed milk producers used plastic made milk containers either during milking or transportation. Similarly, Desalegn (2017) reported that the milk containers were washed with hot water and soap and kept either hanging them on rafts or put on ground. Abebe *et al*.(2012) finding also conforms with this finding in that all farmers from Ezha district of Gurage Zone were used plastic jars as milking utensil. Milk containers such as non-food grade plastic cans, buckets and jerry cans are not recommended in the production of clean milk (Kurwijila, 2006). Aluminum containers are recommended because of not having adhesive properties and therefore easy to clean when compared with plastic containers (Karuga, 2009).

Regarding uses of water types for equipments washing, Haile *et al.* (2012) from Hawassa reported similar results where less proportion of respondents were used cold water. But the same study reported better in using warm water together with detergents (85.6%) to wash milk handling equipment. Unlike this finding Debela (2015) reported that 78% of milk producers use unboiled water to wash equipment, udder and hands while 89% of them did not use detergents/disinfectant. A good hygienic practices leads to the destruction of harmful bacteria and positively contributed to quality keeping of the milk (Kurwijila, 2006). Most milk suppliers (74.3%) were located in a distance between 500 and 1000m meters where as 24.6% were located at a distance below 0.5km, this has its own contribution in reducing growth of microorganism because it takes shorter time to deliver milk to collectors or processer.

### 5.2.3 Perlimnary physicochemical quality of milk

#### 5.2.3.1 Hydrogen ion concentration (pH)

Milk from Bishoftu area farm pooled was significantly higher in PH than the other types of samples. Milk from Sebeta area was lower in pH, whereas Bishoftu was almost similar to the required milk quality standard. Similar study from Shashamene area were reported by Gemechu *et al.*(2015), value better than Sebeta and almost similar with that of Bishoftu's area farm pooled. Higher pH than this finding was reported by Abdulkader *et al.*(2015) ranging between 6.57 and 6.77 from Bangladish sylhet Dairy Farm. According to FAO (1987) pH quality settings those milk below 6.3 is acidic, hence milk from Sebeta fall under this whereas pH of milk in between 6.4 to 6.6 is slightly acidic where Bishoftu areas milk fall in. The relative reduction of pH values in milk may be due to fermentation by lactic bacteria, raw milk storage and transportation to collection site is done at ambient temperature plus lack of cooling (refrigeration) and delayance on delivery aggravate the condition.This is more likely during the dry season and in the afternoon when ambient temperatures are relatively high.

#### 5.2.3.2 Density/Specific gravity/

The specific gravity of milk was affected by difference in site of milk sampled areas .There was a significant difference in specific gravity of the milk among the study areas. The raw milk value of specific gravity in general falls within the ranges of Ethiopian quality standard. The specific gravity of pasteurized milk was significantly lower than the raw milk as well as the Ethiopia quality standard. Similar finding was reported by Gemechu *et al*. (2015) from Shashamene for milk collected from small scale milk producers and dairy cooperative collection center. Abdulkader *et al*. (2015) finding from Bangladish was also reported similar value ranged between of 1.029±0.0025 and 1.032±0.0015.

#### 5.2.3.3 Freezing point

The freezing point of milk is determined primarily to prove milk adulteration with water and to determine the amount of water added (Zagorska and Cipovica, 2013). Similarly, Henno *et al.* (2008) reported that the freezing point of milk is used as one of the quality criteria for insuring high quality milk. The overall mean freezing point of milk sampled from Sebeta and Bishoftu area showed significant differences where the freezing point of milk from Sebeta area(-0.619°C) was significantly lower than that of Bishoftu area(-0.486ºC). There might be adulterations of solutes in milk from Sebeta area milk whereas water adulteration in milk might be high in Bishoftu areas. The frezing points of milk from the study areas was not with in the ranges of Ethiopian standard (ES, 2009) (-0.525-0.550) and -0.540 ºC of OAANC (2005) and FAO (1987).

### 5.2.4 Chemical composition of milk sampled from Sebeta and Bishoftu areas

#### 5.2.4.1 Lactose content of milk samples

Lactose content (4.36%) of Bishoftu, which was lower than content of lactose of Sebeta area(4.91%), was comparable with the findings reported by Haftu and Degnet (2018). Similar study from Shashamene area reported that the overall lactose (4.43±0.06) percent of almost similar with that of Bishoftu area but less than that of Sebeta area finding (Gemechu *et al*., 2015). This study result is greater than that of the overall Lactose % value (4.28±0.08%) reported by Desyibelew and Wondifraw (2019) from Amanuel Town of Ethiopia. In agreement with current finding of Bishoftu area, lactose percentage value for raw milk samples were reported by Belay and Janssens (2014) (4.34±0.13) from different dairy farms of Jimma town. In overall the lactose percent of this study conform to that of European Union quality standards for unprocessed whole milk, which should not be less than 4.2%(Tamime, 2009).The variation between the two sites in lactose content might be due to breed differenc, management practices such as feeding management, and environmental factors that influence the milk composition (Pandey and Voskuil, 2011).

### 5.2.4.2 Protein content of milk samples

The overall protein% value from Sebeta areas fulfill the minimum standard of Ethiopian milk quality values (3.2%) but far higher than that of European Union (EU) quality standards (Raff, 2011) (2.73%). The percent protein (2.9%) content of milk samples from Bishoftu area was less than Ethiopian standard value (3.2%) but higher than that of European Union (EU) quality standards. Almost similar figure with the current finding of Sebeta area was reported by Dehinenet *et al.* (2013) that protein percentage was 3.12±0.32 of milk samples from the Godino and Babogaya. Rehrahie and Yohannes (2000) reported higher value of 5.61% protein for Borana x Friesian from Holetta area. This study result is greater than that of the overall protein % value (2.83 ±0.06%) reported by Desyibelew and Wondifraw (2019) from Amanuel Town of Ethiopia. Almost similar results with Sebeta area finding were reported by Desalegn *et al.* (2017)(3.27±0.15), Debebe (2010) (3.2±0.22), Mirzadeh (2010) (3.2±0.22%) and Belay and Janssens (2014) (3.21±0.06) but greater than this study finding in Bishoftu areas. The lower protein content might be due to deficiency of crude protein in the cow ration.

### 5.2.4.3 Fat content milk samples

The percent fat content of milk sampled from Sebeta area(3.68±0.25) was higher than that of Bishoftu areas(3.59±0.13)**.**The overall values of this study for fat% sampled from Sebeta areas higher than that of the minimum Ethiopian standard values(3.5%) and European Union (EU) quality standards (Raff, 2011) (3.25 %). But the Bishoftu areas value almost similar with that of Ethiopian standard values (3.5%) and but higher than that of European Union (EU) quality standards (Raff, 2011) (3.25 %). However, it is less than the finding of Asaminew (2007) who reported 4.14 fat percent for crossbred cows' milk from Bahir Dar milk shed. Greater result (4.12 ± 0.26%) for overall average value of fat was also reported by Desyibelew and Wondifraw (2019) from Amanuel town of Ethiopia. The current result of fat content from both study sites are less than the report of Alganesh (2016) 3.76% fat content from raw milk collected in peri-urban areas of Ejere, Walmera, Selale and Debre Birhan districts of the central highlands of Ethiopia, Teshome *et al.* (2015) (4.28±0.05) % fat from raw milk of Shashemene town, Southern Ethiopia, and Kunda *et al.* (2015) who reported 3.9% fat from raw milk produced from Lusaka Province of Zambia.

#### 5.2.4.4 Solids not fat content of milk samples

The overall values of this study for solid not fat (SNF)% sampled from Sebeta areas (8.93±0.22) higher than that Bishoftu area (7.93±0.11) and European Union (EU) quality standards (Raff, 2011) (8.25 %). But milk sampled from Bishoftu area had less %SNF than that of European Union (EU) quality standards (Raff, 2011) (8.25 %).the percent SNF content of milk sample from Sebeta area is comparable with the findings of Dehinenet *et al.* (2013) who reported solid not fat (8.88±0.83)% for milk samples collected from Godino and Babogaya. Higher than the current result was reported by Helen (2007) who reported SNF contents of 10.7% for cows' milk in Kombolcha woreda. Almost similar to current study result for SNF (7.77±0.14%) was reported by Desyibelew and Wondifraw (2019) from Amanuel Town of Ethiopia

#### 5.2.4.5 Total solids content of milk samples

Total solid percentage composition of milk from Sebeta area was higher than that of Bishoftu areas. The overall values of this study for total solid % sampled from Sebeta areas less than the Ethiopian standard values (12.8%) but it was in agreement with European Union (EU) quality standards (Raff, 2011)(12.5%). But sample result obtained from Bishoftu area was less than both quality standards. Higher than the current study results was reported by Shibru and Mekasha

(2016) in Sebeta area where overall percentage composition of total solids (12.92± 1.6). The overall mean TS of Sebeta (12.61%) and Bishoftu (11.52%) content obtained in this study is lower than the findings of Derese (2008) and Alganesh (2002) who reported 13.55% and 14.31% for Boran and Horro cows' milk, respectively. Finding reported by Desyibelew and Wondifraw (2019) for TS% (11.89 ±0.40) was greater than that of Bishoftu area finding but less than of Sebeta's finding of this study. The lower average total solids might be due to the practice of adulteration and fat skimming before taking milk to collection points.

#### 5.2.4.6 Total ash content of milk samples

The total ash percentage composition of milk sampled from Sebeta area of this study was higher than that of Bishoftu areas**.** Less than the current finding report for total ash (0.59%) was reported by Shibru and Mekasha (2016) in earlier study from Sebeta area. The ash content (0.74%) obtained in the current study of Sebeta area is comparable with the findings of Dereses (2008) and Assaminew (2007) who reported ash content of 0.73% and 0.74% for milk sampled from Bahir Dar milk shed and west Shoa areas, respectively. However, the overall mean ash content obtained in this study from Bishoft area is lower than the findings of Dereses (2008) and Assaminew (2007). The total ash content of milk samples from Bishoftu area is comparable with the finding of Desyibelew and Wondifraw (2019) who reported ash content of (0.63±0.01) % in Amanual towns but less than that of Sebeta areas result of this study.

### 5.2.5 Physicochemical composition of pasteurized milk

The overall percent chemical composition of milk sampled from pasteurized milk from Addis Ababa supermarkets and shops showed significantly lower milk composition for lactose%, protein%, solid nonfat (SNF)%, total solid% and ash% values than the raw milk sampled from Bishoftu and Sebeta area of this study.The specific gravity of pasteurized milk (1.023±0.001) w as far below the Ethiopian standard (ES) (2009) (1.026 to 1.032).The freezing points of this study (-0.422±0.023) was out of the range of Ethiopia standard (ES) (2009) value (-0.525--0.550). PH value (6.01±0.41) results of this study is similar with study results reported by Zelalem (2010) which was ranging from 5.46 to 6.14 with an overall mean value of 5.87. This study result was in agreement with Zelalem (2010) finding for over all protein (2.57%) and fat (3.05%. But this study results for overall total solid (9.78± 0.47) is far below the finding of Zelalem who reported total solids (11.10%). The result of this study was not conforming to the minimum requirement of Ethiopian standard (ES, 2009) for protein (3.20%) and whole milk fat (3.5%).

#### 5.2.6 Correlation among the different raw milk quality parameters

This finding showed correlation among the different milk quality parameters. Protein content was strongly correlated with ash, SNF, lactose, specific gravity, total solid and freezing point of milk. This correlation appears positive and confirms the rule that the higher protein values, the higher the other components. Fat content of milk was weakly correlated with protein, Ash, SNF, lactose, specific gravity, total solid and freezing point of milk. Specific gravity of milk was strongly correlated with protein, ash, SNF, lactose, total solid and freezing point of milk. Similar study by Ouchene *et al.*(2017) reported the significant correlation between fat and protein; dry matter with fat and defatted dry matter. This study also agrees with Luquet (1985) finding that the milk density (specific gravity) increases with the dry matter content and decreases with the fat content.

### 5.3. Microbial Quality and Food Borne Pathogens in Milk and Milk Products of Sebeta and Bishoftu Towns

#### 5.3.1 Standard plate count (SPC) or total bacterial count (TABC)

There was no variationin total aerobic bacterial count (TABC) in raw milk, ayib (local cheese) and Irgo (local yoghurt) sampled from Sebeta and Bishoftu towns. But there exists difference in TABC within site of study due to raw milk sampled from farm pooled of producer and bulk tank of collectors of Sebeta town. Higher total aerobic bacterial count (7.33±0.09log10cfu/ml) was found on milk samples collected from bulk tank of collectors. Pasteurized milk of this study showed the least TABC count (5.21log10cfu/ml).

Milk produced under hygienic conditions from healthy cows, the acceptable bacterial count level is less or equal to 1 x $10^5$ bacteria per ml of raw milk (O'Connor, 1995; FAO, 2003; EACS, 2006) and standard plate count for America is no more than 3x$10^5$cfu/ml, while the standard for Kenya is no more than 2x$10^6$cfu/ml (Ombui *et al.,* 1995). Nonetheless the TABC of raw milk in Ethiopia was reported to be very high as reported in several earlier studies; Godefay and Molla (2000)(1.9×$10^8$cfu/ml), Mehari (1988)($10^7$-$10^9$cfu/ml),Hailu (1989)(1.7×$10^7$-7.5×$10^7$ cfu/ml), To la *et al.* (2007) (7.4x$10^7$),Tolossa *et al.* (2012)(4.3x$10^7$), Yilma (2012)(9.10 log cfu/ml), Shunda *et al.*(2013)(7.35±0.180 to 7.42±0.272log cfu/m), Dehinenet *et al.*(2013) (1.1x$10^8$cfu/ml),Demiss u(2014) (9.62±0.31 -9.78±0.38 log cfu/ml), Amakelew *et al.(*2015)( 6.88 log10, to 7.54 log10), Faisel and Ahmed (2018)(3.4x$10^8$ cfu/ml and 5.96x$10^8$cfu/ml) and Fufa *et al.* (2019)(8.55 log10cfu/m) to (8.99log10cfu/ml). Unlike this Tegene and Tesfaye (2017) (4.77log10cfu/ml) from around Gondar reported less than this finding.

The current finding is in agreement with almost all earlier studies done on milk from different parts of the country.Thus, this finding results exceeds the national, east African and international hygiene standards for total aerobic bacterial counts. This higher count of TABC wa s an index for hygienic problem either during production or during successive handling till collec tion. Therefore it needs strict follow up and necessary interferance on areas assumed to be high for microbial contamination such as keeping health of animal, personal hygiene of milkier, hygienic practices in farm and milk supply chain. Reduction in total bacterial count were observed in milk sampled from farmers who received training on hygienic milk production and handling (Nebiyu, 2008 and Sintayehu *et al.*, 2008).Therefore giving training for producer and milk handlers on milk hygiene is recommendable to reduce the bacterial load in milk and contamination level in general.

### 5.3.2 Total coliform count

Total coliform counts were significant higher in raw milk samples of Bishoftu's (4.48 log10 cfu/ml) than Sebeta towns (3.72log10 cfu/ml). There was no significant variation in TCC with in Sebeta raw milk but higher in samples of bulk tank milk of collectors than farm pooled milk of producer of Bishoftiu town. Higher TCC in yoghurts of Sebeta town than Bishoftu town. Pasteurized milk samples of this study showed lowest total coli form counts.

The result of the present study is 4.48±0.25 log10 cfu/ml in agreement with the finding of Teklemichael *et al.*(2013)(4.13±0.76) log10 cfu/ml from dairy farm samples of Dire Dawa town and karim and Dey (2013)who reported (3.97±0.13)log10 cfu/ml average coliform counts from raw milk samples by from pakistan, Tadele *et al.* (2016) who reported (4.23±0.14log10) from eastern Ethiopia and Alganesh (2002) who reported (4.15log10(1.4x$10^4$±0.26)) from Guto Wayu districts eastern wolega.

Higher total coliform counts than the present results were reported from different regions of the country like (4.93 to 6.52) log10 cfu/ml by Haile *et al.* (2012) from Hawassa, (5.85±0.483) log10 cfu/ml by Tadesse and Bacha (2014) from Jimma, (5.37 to 5.63) log10 cfu/ml by Amakelew *et al.* (2015) and 6.2±0.42 log cfu/ml from milk vender by Teklemichael *et al.* (2013) from Dawa Chefa District of Amhara Regional state.

Contrary to the current finding, relatively less mean total coliform count were reported for instance, (3.2log cfu/ml) by Samson *et al.*( 2012) on whole milk sampled from Debre Brhan, Selale and Holeta and (3.0x$10^4$cfu/ml) by Dehinenet *et al.* (2013) on raw milk from 6 district of Amhara and Oromia National Regional States. Total Coliform count found in yoghurt of the present study either in Sebeta (4.57±0.35log10 cfu/ml) or Bishoftu (3.35 ±0.23log10 cfu/ml) samples is lower than that of 6.57 log cfu/ml reported from Ergo(yoghurt) samples (Yilma and Faye, 2006) and 6 log10 cfu/ml of Werku *et al.* (2015). TCC of 4.42log10cfu/ml count reported by Zelalem (2012) on Ayib (cottage cheese) was greater than the mean coliform count (4.14±0.40)log10cfu/ml of the cheese in this finding.

European Union standards and UK hygiene and food safety regulation recommended TCC for raw cow's milk for direct consumption to be <100 cfu/ml (Fernandes, 2009; Hickey, 2009) whereas East African Community standards (EACS) (2006) recommended less than 3log10 cfu/ml TCC for good quality raw cow milk.The results of the present study, except for pasteurized milk were greater than standard values sets by EACS, indicating for unhygienic handling of milk. Coliforms are used as indicator microorganisms and their presence suggests a risk that other enteric pathogens may be present in the milk and implies poor hygiene (Fufa *et al.*, 2019). Apart from safety and public health concerns, high contaminations by coliforms results in off-flavours in milk and reduced shelf life of dairy products (Reta and Addis, 2015; Kaindi *et al.*, 2011). This might be due to existence of contamination of the milk during milking and at delivery sites, inadequately cleaned udder of cows, barns and milking utensils, absence or improper cooling systems at farms, collection sites and milk selling areas.

### 5.3.3 Escherchia coli (E.coli)

Escherchia Coli counts had showed variation within and among study sites for raw milk sampled from pooled farm of producer and bulk tank of collectors of Sebeta (3.54±0.07log10 cfu/ml) and Bishoftu (2.96±0.28log10 cfu/ml) towns. Higher in E.coli count of yoghurt sampled from Bishoftu (2.93 ±0.20log10 cfu/ml) than that of Sebeta (1.72±0.25 log10 cfu/ml) town.

The mean value of E. coli count of the present study, except for cheese (1.72 ±0.25 log10cfu/ml) of sebeta and pasteurized milk(1.07±0.21log10cfu/ml), was a higher than standard value (2logcfu/ml)(Centre for Food Safety, 2014). Moreover, the present results' of Bishoftu farm pooled milk was similar with the results on raw milk reported from Cameron, with 79.5% prevalence and mean load of 2.25±1.44 log10cfu/ml (Belli *et al.*, 2013) and (2.58 log cfu/ml) of Mohamed *et al.*(2017) from Djibuti. Lubote *et al.* (2014) finding of 90.67% prevalence contamination and mean count 3.0 x $10^3$(3.48log10 cfu/ml) on milk of producers was comparable with Sebeta's result of this study. Mean E.coli count higher than current results which was $6.8\times10^3$(3.83log10) cfu/ml but with relative lower prevalence rate (64.5%), were repo rted from Malaysia milk samples (Chye *et al.*, 2004). E. coli count less than the present result were reported by Pyz-lukasik *et al.* (2014) ($5.0 \times 10^0$ to $1.1 \times 10^2$ cfu/ml) from raw milk of eastern Poland; and Pourhassan and Taravat-Najafabadi (2011) ($2.1\times10^1$ CFU/ml with 75% prevalence) from raw milk samples of Malayer city of Iran. Moreover, in comparison to this study lesser prevalence of E.coli were reported by Fufa *et al.* (2019) (49.6%) for raw milk samples collected from Addis Ababa; Saba and Adzitey (2015) (49.3%) in Ghana and 17.6% from Mekele Kellamino farms (Faisal and Ahmed, 2018).

According to Centre for Food Safety (Food and Environmental Hygiene Department), 2014 hygiene indicator organisms like E.coli in ready-to-eat food at most should be less than $10^2$cfu/ml. Moreover there are shiga toxin-producing E. coli (STEC) strains which can be transmitted to humans primarily through consumption of contaminated foods having direct contact with animals and their environment (US centers for Disease Control and Prevention(CD C), 2014). Shiga-toxigenic Escherichia coli were also identified in fecal samples from 3 of the 7 farms and in a single raw milk sample of Australian dairy farm environments (Catherine *et al*., 2014). Proper cooking and hygienic food handling can prevent shiga toxin causing bacteria E. coli infections to a large extend. The most effective method for prevention is frequent and vigorous hand washing with warm, soapy water and insuring clean drinking water sources and proper sewage disposal in developing nations (Mead *et al*., 1999).

### 5.3.4 Yeasts and mould count (YMC)

Yeast and mold count were showed significant variation with in study sites and between Sebeta (4.67±0.08) log10cfu/ml Bishoftu (5.48±0.05) log10cfu/ml of raw milk samples. There was no significant difference in yeast and mould count of cheese and yoghurt samples due to difference in study site. Higher yeast and mold count were found on raw milk samples collected from Bishoftu whereas, the lowest yeast and mould counts were identified in pasteurized milk samples.

Comparable results were reported (4.9±0.6log10)cfu/ml for raw milk of individual farms and (4.7±0.52log10) cfu/ml dairy farms of Jima area by Tadesse and Bacha (2014), 4.65 log10 cfu/ml by Haile *et al*. (2012) from milk storage containers of Hawasa farm, 4.461 ± 0.044 log10 cfu/ml Debella (2015) from Yabello district of Borana zone and mean (5.1±0.5log10 cfu/ml of yeast and 3.7±0.6 log10cfu $ml^{-1}$ moulds) by Alebel *et al*. (2013) on raw milk from Jimma.

Unlike the current finding, lower colony count were reported by Amakelew *et al*.(2015) where the average yeast and mould counts at farmer, dairy cooperative and hotel were 0.46 log10, 0.62 log10, and 0.74 log10cfu/ml, respectively. The total yeast and mould count of Cheese observed in the present study of Sebeta (4.93 ±0.09 log10 cfu/ml) and Bishoftu (5.03 ±0.17 cfu/ml) towns are less than that of Zelalem (2012) 8.26 log10cfu/ml over all count on cheese. Yeast and mould count of 8.38 log10 cfu/ml of Ergo (yoghurt) reported by Zelalem (2012) was greater than current finding of Sebeta (4.74±0.19log10cfu/ml) and Bishoftu (4.44 ±0.16 log10cfu/ml) towns. The delivery ways of this finding of the milk and milk products to either collectors or consumers was similar with that of Tadele *et al.*(2016) where raw whole milk and its product delivery was carried out at roadsides ground and in open market, which are dusty and more exposed to wind and road side driven introduction of contaminants. Estimation of yeast and mould counts is also useful for evaluating sanitary practices (O'Connor, 1994) and as Codex Alimentarius (ISO 6611E, 2004) 4 log cfu/ml is the required count for standards of quality. High yeast and mould counts in foods including milk cause spoilage (Gamal *et al.,* 2015). Moreover, some moulds, however, are public health concerns due to their ability to produce toxic substances (mycotoxins), which can be resistance to freezing environments, and causing off odors and flavors of foods, further more may not be easily destroyed during food processing or cooking (Wouters *et al.,*2002 and Herrera, 2001).

### 5.3.5 Staphylococuss aures(S.aures)

S. aures counts were significantly higher in Bishoftu (5.19 ±0.07 log10 cfu/ml) than Sebeta (3.88±0.08 log10cfu/ml) of the raw milk and cheeses samples. S.aures count was highest in farm pooled milk samples. This finding showed higher count in raw milk samples (93%) than products

Earlier study reported comparable prevalence (80%) from Sebeta milk collection center (Ayele *et al.*, 2017), whereas, in Bishoftu's earlier study reported relatively lesser prevalence either on farm pooled (44%) container or in bulk tank (72%) of collectors (Fanta *et al.*, 2011). Comparable mean S.aureus count were reported by Pyz-lukasik *et al.* (2014) from eastern Poland where a counts ranging from (3.20 to 4.71) log10cfu/m and Fatine *et al.*(2012) who reported (5.14log10cf u/ml )from raw milk of Kenitra City of Morocco.

Unlike this finding higher mean count were reported from raw milk of Borana pastoral community with mean (±standard error of mean) log10cfu/ml of different sites as Gololicha [7.3

(0.14)], Debeke [7.24 (0.15)], Dibicha [7.42 (0.09)], Ture Kejima [7.41 (0.11)], Wadye kejima [7.4 (0.12)] and Okicha [7.23(0.14)](Debela, 2015). The higher prevalence of S.aures in raw milk than the products of this study is in agreement with finding of Enquebaher *et al.*(2015) from northern Tigray where significantly higher prevalence in raw milk than the products and that of Catherine *et al.*(2014) finding report of Staphylococcus aureus higher existence in raw milk in 6 of the7 farms sampled (85.71%).

Lesser mean (±standard error of mean) (2.72±1.91log10cfu/ml) were reported from Egypt by Ahlam *et al.* (2014) from government farms.S. aureus can gain access to milk either by direct excretion from udders with clinical or subclinical staphylococcal mastitis or by contamination from the environment during handling and processing of raw milk (Donkor *et al.,* 2007 and Peles *et al.,* 2005). The less count of S. aurus in milk products of this might be conditions typically associated with the development of lactic acid bacteria (LAB) which can produce several substances with antimicrobial activity, such as organic acids, hydrogen peroxide, CO2, and bacteriocins inhibiting multiplication of food borne pathogens that are eventually present in the food (Franciosi *et al*., 2009; Nero *et al*., 2008 and Deegan *et al*., 2006).

Milk samples containing pathogenic bacteria like Staphylococcus aureus can pose a potential hazard for consumer health. Several studies have considered 5.0 log10 CFU/mL as the threshold of concern for *S. aureus* and its concentration more than this are unacceptable as state in FDA (1992), Rho and Schaffner (2007) and Ding *et al.,* (2016). It was also reported that S.aures counts ranging in beteween 5log10 cfu/ml to 9log10cfu/gm recovered from food implicated in food poisoning outbreaks (Nunez *et al*., 1997). According to the reports of Bryan *et al.* (1992) S. aures count greater than $5.6x10^{-5}$cfu/gm found on samples of street vendor food of Pakistan secret staphylococcal enterotoxins. This bacteria can gain access to raw milk from infected mammary glands and animals suffering from sub-clinical mastitis excrete pathogens in milk like *S. aureus* (Singh and Garg, 2011; FAO, 2008; Straley *et al*., 2006). The present result is less than the threshold of concern for S.aureus.

### 5.3.6 Listeria monocytogenes ( L. Monocytogenes)

L. monocytogenes counts in raw milk of Bishoftu (4.52±0.16log10 cfu/ml) was significant higher than Sebeta (4.44±0.15log10cfu/ml) towns but there was significant difference in L. monocytogenes counts of cheese and yoghurt samples in respective towns. Lower counts of L. Monocytogenes was identified on cottage cheese and yoghurt samples whereas higher counts were found from samples of farm pooled, bulk tank and pasteurized milk.

Comparable result of 78.5% on retail milk and 59.9% on fermented milk was reported by Appaih (2012) from Gahana and 60% on cheese by Eyasu *et al.* (2015) from central highlands of Ethiopia.

This findings is higher than prevalence of 60% for bulk tank milk and 30% for yoghurt reported by Muyanja *et al.*(2011) from Uganda; Eyasu *et al.* (2015) reports of 18.9% for raw milk, 40% for pasteurized milk and 5% on yoghurt of Ethiopia; Mengesha *et al.*(2009) 26.6% overall prevalence from ready to eat food (i.e pasteurized milk, cheese, ice cream and cakes) of Addis Ababa, 25.93% prevalence on raw milk by Vanegas *et al.*(2009) from Colombia, 28.6% prevalence on raw milk by Kalmus *et al.*(2015) from Estonia and 41.6% prevalence on raw milk by Al-mariri *et al.*(2013) from Syria. While the only completely safe dose of *L. monocytogenes* is zero, even for healthy people, finding indicated that the probability of invasive disease following exposure to even moderate levels of cells is very low vis-à-vis immunity was not suppressed. Hence, presence of *L.monocytogenes* in food at a concentration of <100 CFU/gm carries a very low probability of causing disease (listeriosis) (Chen *et al.*, 2003; EFSA, 2007 and King *et al.*, 2014).

Raw milk (farm pooled and bulk tank), pasteurized milk, locally processed yoghurt (Ergo) and, except sebeta sample cheese in study areas showed a health risk given their high levels of *L. monocytogenes count*. Moreover, existence of L. monocytogenes in pasteurized milk suggests contamination of either post processing or survival of pasteurization. A controls measure to reduce contamination and its infection includes hygienic practices of dairy husbandry, personal hygien, farm environment mangement and proper pasteurization of milk. Moreover, if there is a case of encephalitis animals should be isolated, its placenta and fetuses plus all in contact materials should aseptically removed and destroyed..

### 5.3.7 Salmonella

The results of this finding fall in the range of most studies findings. In agreement with this finding Kaushik *et al.*(2014) reported that *Salmonella* detection in raw milk ranged in between 0.17 to 28.6%. Higher than current finding were detected in Arusha for *Salmonella 37.33*% (28/75) by (Lubote *et al.*, 2014) and 70% in India (Pant *et al.*, 2013). Salmonella prevalence observed in this study of raw milk was relatively lesser compared to 14% prevalence on raw milk of Ahlam *et al.* (2014) Egypt, 20% prevalence of Tadesse and Dabassa (2012) from raw milk of Jimma, 100% prevalence of Hawaz *et al.* (2015) and Teklemichael *et al.* (2013) from dairy farm (25%) and 50% from raw milk venders of Dire Dawa town. Less than this finding results of prevalence was also reported by Teklemichael *et al.* (2013) for the overall prevalence in milk and milk products which was 1.6% (6 of 384), where for cheese (3.1%), raw milk (2.1%) and yogurt (0%).The result in the present study (9%) prevalence is comparable with 10.76% pooled estimates of salmonella contaminated raw milk in Ethiopia (Tadesse and Gebremedin, 2015). Farm pooled prevalence of this study is almost similar with 6.1% prevalence of salmonella from bulk tank milk of Dakata and western Minesota (Jayaroo and Henning, 2001).

## 5.4 Pasturized milk

The low total aerobic microbial counts in this study might be related to the effect of pasteurization. The mean and standard error of mean of microbial count of pasteurized milk sampled from some parts of Addis Ababa is showed difference in microbial count.Microbial loads of pasteurized milk was significantly lower than the raw milk and other milk products counts of total aerobic bacterial count log10 (5.21±0.14), coliform count log10 (2.74±0.54), Escheria coli log10 (1.07±0.21), Yeast and Mould Count log 10(3.64±0.19), Staphylococcus aures log10 (1.91±0.46), Listeria monocytogenes log10 (4.60±0.65)cfu/ml.

Results of this finding total aerobic bacterial count (5.21±0.14) log10 cfu/ml was comparable with the finding of Nato *et al.* (2016) (5.64±3.19) log10 cfu/ml. Higher than this finding of TABC were reported by Yilma (2012) (7.28log10), (Anteneh, 2015) who reported in the range of (6.36 to 7.1) log10cfu/ml from Addis Ababa and Mohammadreza *et al.*(2016) who reported 7.85 log10 cfu/ml for pasteurized milk from teran of Iran. Unlike this finding lesser total aerobic bacterial count (4.43±0.17log cfu/ml) was reported by karim and Dey (2013) for pasteurized milk from Bangladish. Results of the pesent finding was not fulfil quality standards of our country and that of different countries standards sets.

The coliform bacteria are supposed to be absent for fecal coliform or not more than log10cfu/ml for none fecal coliform (QSAE, 2009 and Yilma, 2011) in pasteurized milk as it is assumed can't survive the pasteurization temperature but result of this study for total coliform count of pasteurized milk was (2.74±0.54) log10cfu/ml which is above the standard. Finding reported on coliform count by different researchers on pasteurize milk was in agreement with this one where 2.87 log10cfu/ml by Yilam (2012), fall within finding ranges of Anteneh (2015)(1.18 log10 to 3.28log10cfu/ml) and Tamrat (2018) (1.7 log 10 to 4.36log10cfu/ml) reported from Addis Ababa. Greater than counts of this finding were reported by Dealegn (2015) (4.08log10cfu/ml to 4.84log10cfu/ml) from Kenya and Anderson *et al.*(2011)(4.13log10( 1.34 x $10^4$ )cfu/ml from Ja maica. Unlikely lesser result was reported by Wanjala *et al.*(2017)(0.1log10) and Nato *et al.*(201 6)(1.02±2.6log10) from Nairobi Kenya;Kerim and Dey(2013) [(2.08log10 cfu/ml or(1.2x$10^2$)]cf u/ml) and[1.11log10 cfu/ml or (1.3x 10 cfu/ml)]of sahah and Ara (2012) from Bangladish. Results of this finding were greater than the different countries standards for quality requirement of pasteurized milk (≤10cfu/ml of ES, 2009; ≤ 5cfu/ml or gm for EU and ≤ 10cfu/ml USFDA, 2011). Though it wasn't bring it below quality standards, pasteurization has showed significant reduction in microbial counts, this might be due to its high count in raw milk was above the level to be treated to low count or it can be in agreement with Kiiyukia (2003) inferrence, in that the presence of coliform in heat treated milk is an indicative of a defective pasteurization process or post pasteurization contamination.

The mean yeast and mould count of pasteurized milk of this study is (3.64±0.19) log10cfu/ml which is less than the (6.68±0.24) log10cfu/ml reported by Korma *et al.* (2018) in pasteurized milk sample collected from Hawassa city supermarkets and dairy shops.

Pal *et al.* (2012) mention as an important notice that pasteurized milk can also harbor the pathogenic bacteria such as E. coli, L. monocytogenes, Salmonella spp., and S. aureus. The mean S.aures count of pasteurized milk in the present study is (1.91±0.46) log10 cfu/ml which is with 50% prevalence was much higher than the result (0.10log10cfu/ml) reported by Wanjala *et al.* (2017) from pasteurized milk marketed in and around Nairobi region of Kenya. Unlike this finding higher count of S. aures (6.89±0.27) log10 cfu/ml was reported by korma *et al.* (2018) from Hawassa. However earlier study by Ayele *et al.* (2017) from Sebeta reported for absence of S.aureus isolate from pasteurized milk.

The mean *E.coli* count (1.07±0.21log10) with prevalence of 33.34% in the present study is similar with the finding of Anteneh (2015) who reported 1.28 log10 (1.9x10 MPN) in Addis Ababa. Results on E.coli, which was, much higher count 4.34log10 cfu/ml than the current finding were reported by Silva *et al.*(2010), on the other hand, 18.5% prevalence of Hawassa was far below this study (Korma *et al.*,2018). Similar finding on pasteurized milk from selected supermarkets in developing country like Jamaica reported unacceptable level of Escherichia coli in their most samples (Anderson *et al.*, 2011). This finding is in agreement with Kiiyukia (2003) inference, in that the presence of E.coli in heat treated milk is an indicative of a defective pasteurization process or post pasteurization contamination.

The mean Listeria Moncytogen count in pasteurized milk of this study is 4.60±0.65log10 with 83.3% prevalence rate is much higher than 40% prevalence of Seyoum *et al.*(2015) and 2.8% prevalence from Hawasa (Korma *et al.*, 2018). However, it is less than Dalton *et al.*(1997) finding reported for post pasteurization contamination due to poor sanitation practices followed by temperature abuse in unopened containers to reach 8 to 9 log cfu/ml of Listeria monocytogenes. Unlike this study lower result was reported for unfinished UHT at milk storage temperature of 35°C for 6 hours time result in Listeria monocytogenes counts of 100CFU/mL (Siti *et al.*, 2018).

Pasteurized fluid milk is most susceptible to microbial or other flavor defects related to raw milk quality. Thus, the typical microbial defects in pasteurized milk may include acid, malty, bitter, coagulated, rancid, unclean, fruity and fermented (Alvarez, 2009). Bacterial defects typically become apparent in raw milk and processed dairy products only when bacterial numbers are high, generally >1,000,000 cfu/mL in raw milk to be processed depending on the specific microorganisms present and their metabolic states (Boor and Murphy, 2002).Worldwide standardized pasteurization practices would be an effective first step in eliminating or reducing the levels of many spoilage microorganisms (Ledenbach and Marshall, 2009).Hence different standard authorities recommended standards to be below 5 log10 for total aerobic bacterial count (Quality and standard authority of Ethiopia (QSAE), 2009; US Pasteurized Milk Ordinance (PMO); FDA, 2013) and 4.48 Log10cfu/ml according to East African Standard-Pasteurized milk specification (EAS 69:2006) and not more than 1log10 for total coli form count (QSAE, 2009).Results of this study is above the standards. Based on the results of this study pasteurization has showed a significant reduction in microbial count in milk but when it is compared with setted standard of qualities, pasteurized milk may not necessarily a complete safer than its raw milk. Therefore, in addition to delivery/collection site monitoring, furthermore, processors need to increase severity of pasteurization regime based on microbial quality of milk. Proper cleaning and sterilization of plant equipment, surfaces and aseptic packaging is necessary to avoid post-pasteurization contamination.

Regular based microbiological monitoring of the milk and dairy products should be conducted to keep the products safe to the consumers; provision of incentives to milk suppliers such as quality based payment to encourage delivery of good quality milk for processing. The substandard quality of pasteurized milk observed in the present study may result in the out break of various food borne illness when pasteurized milk is consumed as it was concluded by (Jackson *et al.*, 2011, Koch *et al.*, 2010 and CDC, 2008).

# 6. CONCLUSION AND RECOMMENDATIONS

## 6.1 Conclusion

Twelve years milk yield records of cows at Holleta Agriculture Research Center dairy farm revealed that milk yield was significantly affected by season, cross of exotic blood level, dam parity and lactation stages. Results of high milk yield and fat% with low protein% were observed in dry season. An increase in exotic blood level and dam parity results in an increase in milk yields. Season, parity and lactation stage significantly influenced protein percent of milk whereas fat% of milk was more significantly affected by season. The data analysis results for percent fat, protein and total solids fulfill Ethiopian, European aswell as FAO quality standards.

From results of physicochemical analysis of raw and pasteurized milk samples of this study, Sebeta area milk samples showed high percent for fat, solids not fat (SNF), total solid and ash values than that of Bishoftu. Moreover, mean value for protein, fat, solids not fat percent of Sebeta milk was within the range of Ethiopian standard values, but for Bishoftu area, except for fat percent all components were below the standards. Whereas, when it is compared with that of FAO requirements only Sebetas fulfills the requirements, for solids-not-fat, all the rest either from Sebeta area or Bishoftu area were bellow the FAO standards.

The microbial count for cheese, yoghurt, raw and pasteurized milk were above the acceptable limits of quality standards. Comparatively the microbial counts from milk of Sebeta area were lower than Bishoftu area. In overall, almost in most investigated types microbial count, the result found was above the national, east African community and in international quality standards. Higher count of microbial in raw milk than in cheese, yoghurt and pasteurized milk samples. The high microbial counts and existence of pathogens in the milk suggest the existence of contamination from various sources. Hence, it necessitates to follow proper barn and dairy equipment cleaning, animal health cares, strict hygienic practices in dairy farms, at milk collection sites and in dairy processing plants to minimize the microbial contamination of the milk in the study area. Therefore in general, for ensuring high quality raw milk production under good hygienic conditions and application of control measures are significantly important to protect human health.

## 6.2 Recommendations

The physicochemical quality in overall was not far away from the quality standards. It is recommended to monitor strictly at delivey point to reduce adultration.There was a major problem of safety in hygienic milk production, this may be due to various factors, Therefore it is recommended that

- Training and guidance should be given to farms' owners and their workers on quality milk production.
- Avoiding of open space(road side) collection of milk, facilitating dust proof transportation and setting cooling systems at milk collection centres
- Implementation of Hazard analysis critical control point (HACCP) system is more comprehensive to identify point at which the milk and its product more subject to bacterial contamination.

Moreover, the domestic market is characterized by inadequate regulatory and market incentives for milk safety and quality.Therefore, bringing all dairy stake holders together for quality milk production and implementation of quality payment programs is recommended. Some of pathogenic organisms in the milk of study areas were above the thresholds of concernand may be harmful to human health. Therefore, frequent inspection and put measures in place to ensure its comformity with the safe quality standards set. The poor microbial quality of pasteurized milk de manding more attention for corrective actions to ensure the public health safety.

## Future research area

- This study covers commercialized milk and milk products coming from urban and peri-urban sources only, except Ayib (cheeses), representsing about the 2% milk sources in country future research area will be better if it consider the rural area milk and milk product sources.

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# 8. APPENDICES

**Quality and Safety of Raw and Pasteurized Cow Milk Produced and Marketed in central highlands of Ethiopia**

Dear respondent:

This questionnaire is used to get back ground information concerning milk production, handling and distribution practices amongst milk collectors and milk vendors. The information was used for graduate research purpose at Addis Ababa University. I appreciate your kind cooperation in completing this questionnaire.

Instructions:

To full fill the questionnaire you are kindly requested to follow the following rules. In the space provided, give brief answers. For questions that have multiple choice select one or multiple of them and encircle on your choice/choices

District…………………………Division............................................Location......................

Sub-location.....................................Name of milk collection centre……………………………

Name of DFBA ………………………………….

Weather on date of sample collection: hot/cold/dry/wet…………….

1= Hot 3= Dry 2=Cold 4= Wet

**A) General information**

1. Name of Respondent .................................................Date of interview..............................

| | | |
|---|---|---|
| **Respondent's position in the household** (code) **1 = Head** | **3 = Daughter** | **5 =Employee manager/** |
| **2 = Spouse** | **4 = Son** | **6=Other(specify)_______** |

**House hold head details**

| | |
|---|---|
| Sex [code: 1 = Male, 2 = Female] | [______] |
| Age (years) | [______] |

| | |
|---|---|
| Years of farming experience (years) | [______] |
| Number of years of schooling (years) | [______] |

2. Is there anyone in the household who has had any training on milking and/or milk hygiene /handling/testing?

Yes…….No…….

If yes, what is the duration of the training on milking hygiene/ handling/ testing?

1) No training 2) Up to one month of training

3) Between one month and 6 months of training 4) More than 6 months of training

3. Where and in what year did the training take place?.........

| | | |
|---|---|---|
| **1 = Head** | **3 = Daughter** | **5 = Hired manager/employee** |
| **2 = Spouse** | **4 = Son** | **6 = Any of options 1-5** |

4. Who does the milking? …….

5. If the milking is done by someone other than the household head, provide the following information about the milker.

| | |
|---|---|
| Sex [code: 1 = Male, 2 = Female] | |
| Age (years) | |
| Years of farming experience (years) | |
| Number of years of schooling (years) | |

6. Specify milking and/or milk hygiene/handling/testing that you are aware of........…

1. = None 5. = Boiling

2. = Organoleptic test 6. = Hygiene Management

3. = Lactometer 7. = Dairy Standards

4. = Alcohol test 8. = Clean milk handling

9. = Milk preservation 10. = Others (Specify)______

**B) Herd information**

7. Herd size…………………

Number of lactating cows in the herd………………

Number of Heifers in the herd…………………

Number of calves in the herd…………………

Number of dry cows in the herd…………………

8. Number of milking /day…………...

**C) Hygiene information**

9. Do you wash your hands before milking? (1)Yes… (2)No…If you wash, what do you use?
(1)Water alone; (2) Water + soap/disinfectant; (3) other (specify) ______

10. If you wash your hands, do you dry them before milking? (1) Yes….. (2) No……..
If you dry your hands, what do you use?
(1)Newsprint; (2) Disposable paper towels; (3) Re-usable cloth; (4) other, specify ______

11. Do you wash your cow's udder before milking? (1) Yes (2) No..If yes, when do you wash it?
(1) Cleaned before milking only (2) cleaned after milking only
(3) Cleaned both before and after milking

12. If you clean the udder, what do you use (1) Udder cloth……. (2) Disposable towel…

13. If the answer in udder cloth, do you use a separate one for each cow? (1)Yes…. (2)No……

14. If you use the udder cloth, how often do you wash it?
(1)Daily……………………. (2)Weekly………………… (3) Never…………….

15. How do you wash the udder cloth?
(1) With warm water………….. (2) With warm boiled water
(3) With cold unboiled water……….. (4) With cold boiled water

16. Do you use a sanitizer when washing the udder cloth? (1)Yes…. (2)No……
If yes, what type of sanitizer do you use? (1) Hypochlorite…………. (2) Iodophore
(3) Other (specify)…………………..

17. Do you use milking cream? (1) Yes……….. (2) No…..……..

18. Do you use teat dipping after milking to prevent mastitis? (1) Yes… (2) No ………

19. What type of milk container do you use?
(1)Plastic……………… (2)Aluminum………………… (3)Other……………………

20. How often do you wash the container?
(1)Before every use (2) After every use (3), before and after every use

21. How do you clean the container?

(1)With cold water alone (2) with hot water alone… (3) With cold water and soap….(4)With hot water and soap……… (5) With detergent and water (6)Others (specify)……

22. What is your source of water?

(1) Piped/ tap……(2)River/ stream……(3) Community ground pump…(4)Roof catchment (rain water)……(5)Private ground pump/well……(6)Other (specify)…………

23. How do you store the milk containers after cleaning?

(1) On tables … (2) Hanging them…… (3) On the ground… (4) Other (specify)

24. Do you cool the milk before sale? (1)Yes…. (2)No……

25. How much milk do you:

a) produce in a day?................

b) keep for home consumption in a day?.........

c) Sell in a day?..................

26. How far is the milk collection centre?.......... and how do you transported the milk to collection station?

A. On foot B. By truck C. By bicycle D. By motorbike

27. How long does it take you to transport the milk to the collection centre/ dairy processing?

(1)< 30 minutes…… (2) 30-45 min…… (3) 45- 60 min… (4) > 1 hour…….

28. Do you house your cows? (1)Yes…. (2)No……

If yes, what is the floor of the cow shed made of?...........................

1= Dirt 3 = Wood 5= Other (Specify)

2= Concrete 4= Stones

29. Does your cow shed have bedding? (1)Yes…. (2)No……

If yes, what type of bedding?..................

1= Straw 3= Wood shavings 5= Soil

2= Grass 4= Dry Maize Stalks 6= other (Specify)……………..

30. How often do you clean/ remove manure from the shed?.....................

1= Daily 3= Monthly 2= Weekly 4= Other (specify)………………….

**D) Milk composition and hygienic quality**

31. Have you ever used antibiotics to treat your animals? (1)Yes…. (2)No……

If yes, are you aware of the antibiotic withdrawal period? (1)Yes…. (2)No……

If yes, did you observe this period? (1)Yes…. (2)No……

32. Are you aware of some of the compositional parameters in milk? (1)Yes…. (2)No……

If yes, are you aware of how to influence the compositional quality of milk? (1)Yes… (2)No..

33. Are you aware of how the quality of your milk compares to others? (1)Yes…. (2)No

If yes, is it (1) above average (2) average (3) below average?.....................

34. If the cooperative introduced higher payments for those delivering milk of bacteriological and compositional quality, would you support this? (1)Yes…. (2)No……

35. Does your milk get spoilt before delivery? (1)Yes…. (2)No……

If yes, how many times has it spoilt in the last week?

36. Has your milk been rejected by the cooperative in the last one month? (1)Yes…. (2)No……

If yes, why was it rejected?......

1. = Low fat 3=Abnormal colour 5=Failed Alcohol test

2. = Low Density 4= Abnormal smell 6= Dirt 7.Other (Specify)

37. Do you do any milk test before delivering milk to the collection centre?

(1)Yes…. (2)No……

If yes, which are these tests?..........

1= Alcohol test 3=Density Test

2= Clot on boiling test 4. Other (Specify)………..

38. What type of feed do you give your animals?...............

1= Concentrates Only 2= Napier only 3= Free range

4= Grass and other forage 5= Mixture of Concentrate and other Feed

**MASTITIS RELATED QUESTIONS**

39. Who primarily milks the lactating cows? (Multiple choice)

a) Myself b) Family member only

c) External employees d) both e) others (specify)……

40. Is it primarily men or women who milk cows?

a) Male b) Female c) Both

41. List IN DETAIL all the steps undertaken when milking one of these cows, starting from the point of approaching the cow. Pay attention to not forget any steps List…..

42. You said that you do wash your hands, what do you use to wash your hands?

a) Water only b) Water with soap c) Water with a disinfectant and soap

43. You said that you do clean udder and/or teats of the animal, what do you use to wash teats?

a) Warm water only  b) Cold water  c) Water with a disinfectant

44. Which milking technique do you use? (Multiple choice)

a) Hand milking  b) Machine milking

45. If hand milking, which technique do you use?

a) Stripping (Pulling the teat)  b) Squeezing(full hand milking) Action  C. both

46. Can you recognize if your cow has an infection/ a problem in the udder?

a) Yes  b) No

47. If yes, how do you detect? (Tick)

a) Change of colour of the udder/teats  b) Udder feels warm than usual

c) Changed consistency of the udder  d) Changed size of the udder

e) Presence of visible lesion on the udder  f) Udder veins are engrossed

g) Changed milk consistency and colour  h) Others(specify)……

48. If yes, do you milk animals with udder problem?

a) Yes  b) No (go to question 82)  c) Sometimes

49. If yes, when do you milk the animal(s), before or after the health ones?(options)

a) Before the health animals  b) After the healthy animals

c) Others (specify)…………………………………………………………..

50. What do you do with the milk obtained from cows with udder infection?

a) Discard  b) Consume in the your household  c) Sale to the market

d) Feed to calves  e) Others (specify)……………………

**GENERAL ZOONOSES EXPOSURE PRACTICES**

51. What do you do with milk from YOUR cattle herd?(multiple choice- mandatory)

a) Consume within the family  c) Sell to local businesses (restaurant, hotels schools)

b) Sell to milk vendors  d) Sell to milk processing company

e) Sell to neighbours and members of the community  f) Other (specify) ………

52. What is the type of milk collection/storage container used?

a) Plastic container  b) Metal can (almunium)

c) Glass bottle  d) Wooden/pot

53. Was the milk stored under shade for a time being ?

a) Yes b) No

54. Do you store the milk before selling? A. Yes B. No If yes where?

A. In a refrigerated tank B. In an open container

C. In a closed bulk tank D. If other- please indicate:

55. At what time did the milk milked? (Milk storage duration)

a) Less than 2 hrs b) between 2 – 6 hrs

c) Between 6 – 12 hrs d) more than 12 hrs

56. When animal is sick, what do you do with the milk?

a) Don't milk the animal c) consume it in the family

b) Sell the milk d) use it for the calves e) other (specify)…………

57. And if the sick animal is treated with a medicine, what do you do with the milk?

a) Don't milk the animal c) Milk the animal and discard the milk

b) Sell the milk d) Consume it in the family

e) Use it for the calves f) Other (specify)………

58. For how long do you discard the milk (withdrawal periods)……..days

59. Do you consume raw milk?(Mandatory-single choice)

(Note: Raw being unprocessed milk, NOT BOILED, not pasteurized or homogenized)

a) Always (regarded as yes) b) Sometimes (regarded as yes) c) No

60. Do you consume milk products made from raw milk?(Mandatory)

a) Yes b) No

61. If yes, which ones?(multiple choice)

a) Yogurt b) Fermented milk c) Ghee d) Cheese e) All milk products

f) Others (specify)………………………………………………

62. How do you handle an animal that is close to die or dies (dead one) on the farm?

a) Bury (after exitus) b) Burn (after exitus) c) Slaughter and sale and/or eat the meat

d) Others (specify)……………………………

63. How do you dispose the manure from the herd?

a) Do not dispose (Leave on animal house) b) Use in own crop farm

c) Dispose by the road side d) Use it for biogas production

e) Sale f) Others (specify)................................

**Questionnaire for Milk Vendors**

1. From where do you get milk? Dairy farms Street sellers
2. Do you boil the milk before you sell? A.Yes B. No
3. Do you frequently clean the place where you sell the milk? A. Yes B. No
4. Is there any contact between your hand and the milk during selling?

   Yes No
5. From question number 6, is this container cleaned/disinfected regularly?

   A. Yes B. No
6. In what type of container is the milk collected?

   A. Gourd B. Metal container made of aluminum

   C. Clay pot D. plastic container E. Others (if any)
7. Where do you store the milk before selling?

   A. In the container at room temperature

   B. In a container kept at a refrigerator

   C. If other places please indicate:
8. How often do you monitor the milk hygiene?

   A. Some times B. Always C. Not at all
9. How is milk transferred to the vending site?

   A. By motorbike B. By bicycle

   C. By truck D. Labour
10. To whom do you sell the milk? A. Hotels

    B. Individual consumers C. All of them