HAWASSA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

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DEPARTMENT OF CHEMISTRY

PHYSICAL PROPERTIES AND CHEMICAL COMPOSITIONS OF PASTEURIZED COW MILK MARKETED IN ADDIS ABABA, ETHIOPIA

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HAWASSA, ETHIOPIA

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A MASTER THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE, SCHOOL OF GRADUATE STUDIES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN CHEMISTRY

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NOVEMBER, 2024

HAWASSA, ETHIOPIA

DECLARATION

I hereby declare that this MSc thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis/dissertation have been duly acknowledged.

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We, the undersigned, members of the Board of Examiners of the final open defense by Wubedel Teka (ID. № PGChemK/015/11) have read and evaluated his/her thesis entitled **"Physical Properties and Chemical Compositions of Pasteurized Cow Milk Marketed in Addis Ababa, Ethiopia''** and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry.

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LISTS OF ACRONYMS/ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
CODEX	Codex Alimentarius
ESL	Extended shelf-life
FAAS	Flame Atomic Absorption Spectrometry
FAOSTAT	Food and Agriculture Organization of the United Nations Statistics Division
HTST	High Temperature Short-time
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LTLT	Low temperature long-time
MDL	Method Detection Limit
MFG	Milk Fat Globules
NANA	N-acetyl neuraminic acid
PET	Polyethylene
SNF	Solid-not-fat
TAG	Triglycerides
TTA	Titratable acidity
UHT	Ultra-high-temperature

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ABSTRACT

Milk represents an important intake in a typical diet due its high nutrient and mineral content. Consequently, milk must be of satisfactory quality in order to protect the health of the

community. This study investigated Physical Properties and Chemical Compositions of Pasteurized Cow Milk Marketed in Addis Ababa, Ethiopian. Seven locally available pasteurized cow's milk samples were collected and codes were given. Physical Properties and Chemical Compositions were determined following standard methods of analysis. The mean values of pH, moisture, total solids, total ash and titratable acidity were in the range of 5.81–6.42, 84.78–91.91%, 8.09–15.16%, 0.546–0.739%, and 0.228–0.411%, respectively for the seven pasteurized milk samples. The determination of minerals in pasteurized cow's milk samples were carried out after optimization of the digestion of milk, which were found to be (5.0 mL HNO_3 (70%): 3.0 mL $HClO_4$ (70%) volume ratio of reagents, 200 °C digestion temperature and 2:00 hours digestion time for the digestion of 5.0 mL of milk samples. The mean concentration of Ca, Mg, Cu, Mn, Ni, Co and Cr were in the range of 1041.00–1609.00 mg/L, 72.00–122.00 mg/L, 0.0351–0.0819 mg/L, 0.0511–0.1019 mg/L, 0.0601–0.0684 mg/L, 0.0518–0.0814 mg/L and 0.0531–0.0809 mg/L, respectively for the seven pasteurized cow's milk samples. In this study, the concentration of Ni was also found to be below the detection limits for pasteurized cow's milk samples such as AA/B1, AA/B4, and AA/B6. However, the concentration of Pb was found to be below the detection limits for all pasteurized cow's milk samples. Results indicated that there were significant differences ($P<0.05$) in most of the evaluated physicochemical parameters and minerals content among the seven pasteurized milk samples. The accuracy of the method for the determination of the selected elements was evaluated through recovery experiment and it lied within the range 98.0% to 114.1%, which is in the acceptable range (80–120 %). Generally, the levels of the selected physicochemical parameters and several metals were mostly found above the safe limits adopted from international food standards like WHO/FAO.

Key words: Pasteurized milk, physical properties, chemical composition, Addis Ababa

1 INTRODUCTION

1.1 Background of the Study

Milk is a complex mixture of bioactive substances that play a crucial role in promoting the growth and development of mammalian infants [1]. It is the primary source of nutrition for young mammals before they are able to digest other types of food [2]. It is an excellent source of calcium, vitamin D, riboflavin, and phosphorus and a good source of protein, potassium, vitamin A, vitamin B-12, and niacin [3]. It also provides a moderate amount of magnesium, a smaller amount of zinc, and very smaller amounts of iron and copper to the body [4].

Mammals such as cows, buffalo, goats, sheep, reindeer and camels are utilized for their milk production. The milk from such mammals are used for different nutritional purposes; for example, feeding young ones and preparation of some nutritional products such as milk cream, butter, yogurt, sour milk, etc. [5].

Milk and its products are the main constituents of the human daily diet because it provides all macronutrients (such as proteins, lipids, and carbohydrates) and all micronutrients (elements, vitamins, and enzymes), especially for vulnerable groups such as infants, school age children as well as old aged peoples [6]. It is the only source of nutrients during the first months of a baby's life and the diet of growing children contains a high proportion of milk and its products. Thus, the constituents of milk and its products are essential to promote the growth and maintenance of human life during three periods of life: childhood; providing protein, minerals, and fat to support the body's development, during adolescence; offering conditions for rapid growth building consistent muscle, bones and endocrine [7] and for elderly people it represents a source of calcium essentially to maintain the integrity of bones. Thus, milk and its products should be free from contaminants such mycotoxins, dioxins, heavy metals like lead, and cadmium due to their toxic effects on humans. Moreover, the quality of milk sold in the markets should be assessed to prevent adulteration of milk and ensure the quality and safety of milk sold to the consumers. Thus, this study was undertaken to address these issues, and hence the objective of the study was to determine some physical properties and chemical compositions of pasteurized cow's milk samples marketed in Addis Ababa, Ethiopia.

1.2 Statement of the Problems

Milk must be of satisfactory quality in order to protect the health of the community. Milk represents an important intake in a typical diet due its high nutrient and mineral content. Many dangerous element or compound, such as metal and metalloid, accumulate in food chain. In addition, their concentration in the environment grows with the increase of urban, agricultural process and industrial emissions. One of these almost ubiquitous presences of some metal pollutant, especially cadmium and lead, facilitate their entry into the food chain and hence increase the possibility of them having toxic effects on humans and animals, although heavy metals have industrial uses, their potential toxicity for people and animals is the subject of several study. For some element the effects are accumulative and it is necessary to control them level in consumed milk [8]. Furthermore, producers or milk processing industries might sell adulterated milk that compromises the compositional and processing quality and the hygienic and nutritional quality of the milk. The society consumes the pasteurized cow milk of Mama, Loni, Zagol, Harme, Emit, Shola, and addey. But the quality of milk has not been assessed based on physicochemical parameters and mineral composition. To mean that no more analysis was made on physicochemical parameters and mineral composition in cow's milk related to their use and negative impacts to human's health. The quality and composition of pasteurized cow's milk is affected by various factors such as improper handling and storage practices, contamination during processing, cow's feed sources, drinking water and agricultural process. These factors can impact the physicochemical parameters and mineral composition of the milk, leading to potential changes in taste, nutritional values and safety. Therefore, it is necessary to determine and monitor the physicochemical parameters and mineral composition of pasteurized cow's milk available in the local market.

1.3 Objectives of the Study

1.3.1 General Objective

The main objective of this study was to investigate some physical properties and chemical compositions of pasteurized cow's milk marketed in Addis Ababa, Ethiopia.

1.3.2 Specific Objectives

- To determine physicochemical properties such as pH, moisture, total solids, total ash, and titratabe acidity in pasteurized cow's milk samples.
- To determine the concentration of selected essential metals (Ca, Mg, Cu, Mn, Ni, and Co) and toxic metals (Cr and Pb) in pasteurized cow's milk samples.
- To compare the values of the physicochemical properties and mineral compositions of the pasteurized cow's milk samples studied among the pasteurized cow's milk brands.
- To compare the values of the physicochemical properties and mineral compositions of the pasteurized cow's milk samples with national and international standards.

1.4 Significance of the Study

- The study provides insights into the quality of pasteurized milk sold out in the local market in Addis Ababa, Ethiopia.
- The study aids regulatory bodies to ensure the quality and safety of pasteurized milk sold out to the consumers.
- The findings may help milk producers and dairy processors to improve the quality and safety of their products according to national and international standards.
- The study provides a basis for further research regarding milk production, safety and quality.

1.5 Limitations of the Study

The limitation of this study was lack of hollow cathode lamp for the determination of phosphorus, iron, zinc which are an important indicator of nutritional content of milk and cadmium which are important indicators of contamination of the milk samples. Furthermore, some of the physicochemical parameters were not done due to lack of instruments, apparatus, and reagents. The other limitation was that only few samples were taken from each brand of milk available in the local market in Addis Ababa, Ethiopia due to insufficient funds for the research.

2 LITERATURE REVIEW

2.1 Introduction

Milk is a wholesome food and represents an important constituent of the human diet (especially for infants, schoolchildren and the elderly) as it contains nutrients that are essential for growth, bone development, immune function and other important physiological functions [9,10]. It provides an excellent source of all the nutrients and its nutritive value has long been recognized; Indeed, it contains both macronutrients (proteins, carbohydrates, and fat) [1, 6, 11] that contribute to its nutritional and biologic value and micronutrients represented by minerals and vitamins, which play an important role in the body's various vital functions [12–14]. The term "milk" is associated with cow's milk (bovine species) since it is by far the principal type used throughout the world and it represents 83% of world's milk production [15, 16]. However, other animal species are utilized for their milk production, including buffalo (in India, China, Egypt, and the Philippines), goats (in the Mediterranean countries), reindeer (in northern Europe), and sheep (in southern Europe), whose properties are arousing increasing interest, for some beneficial effects on human health [17, 18].

Milk and dairy products such as milk, yogurt, cheese, butter, ice cream etc., are one of the main nutrient classes that your body requires [19]. Compared with other foods, milk offers a cheap and valuable source of vitamins and minerals [20]. Micronutrients are important for human health; Indeed, their deficiency is a cause of malnutrition, which primarily affects the health of the most vulnerable groups in the population, such as kids and pregnant women, especially in poor countries. Some important micronutrient deficiencies with adverse health effects involve vitamin A, vitamin D and calcium [21].

2.2 Physical Properties of Milk

Milk's physical composition was an important parameter, which indicates the nutritional qualities of milk [22]. Milk has unique physical properties, which are used as quality indicators. The principal physical properties of milk include its specific gravity and density. Milk is heavier than water. The density of milk varies within the range of 1.027 to 1.033 kg/cm^3 at 20 °C. The density of milk is often used for quality tests mainly to test for the addition of water to exploit or remove

the cream. In addition, the density of milk is used to estimate the solids content, to convert volume into mass and vice versa.

The specific gravity of cow milk varies from 1.018 to 1.036 and of buffalo milk from 1.018 to 1.038. The specific gravity of milk is decreased by the addition of water, the addition of cream (fat), while the removal of fat and reduction of temperature increase the relative density of milk. Generally, normally milk incorporates a relative density between 1.027 and 1.035 with a mean value of 1.032 at 16 °C [23]. High-quality milk should be white in appearance, don't have any objectionable odors, and be freed from abnormal substances like pesticides, toxic heavy metals, added water or antibiotics, and antiseptic residues [24].

Freshly drawn milk has a pH value in the range of 6.5 to 6.7 and contains 0.14 to 0.18% titratable acid calculated as lactic acid. There is no developed acidity in freshly drawn milk, the slightly lower than the neutral pH being attributed to the presence of carbon dioxide, citrate, casein etc. Treatable acidity is set within the dairy industry mainly for two reasons: (a) to test the freshness of milk and milk products and (b) to regulate the manufacture of cultured (fermented) dairy products. The initial acidity of milk from individual cows varies within the range of 0.08–0.25% carboxylic acid but the titratable acidity of fresh bulk milk seldom falls outside the range of 0.14–0.16% [25]. The freezing points of cow and buffalo milk vary from -0.512 to -0.572 ºC and from -0.521 to -0.575 ºC respectively. Freezing point of milk is mainly used to determine added water. The boiling point of milk is 100.17 ºC.

2.3 Major Milk Components

Milk can be considered a source of macro- and micronutrients, together with bioactive substances, and also contains a number of active compounds that play a significant role in both nutrition and health protection [26]. The chemical composition of Milk varies depending on individuality of the animal, breed variation, nature and quality of the animal feed, stage of lactation, the quarter of the udder of the animal from which milk is drawn, genetics, physical and environmental factors such as seasonal changes, weather [27, 28]. Tables 2.1 summarize the proximate composition, selected vitamin and mineral composition of milk of different mammals.

Table 2.1: The proximate composition, selected vitamin and mineral composition of cow, buffalo, and goat milks (per 100 g of milk)

	Cow		**Buffalo**		**Goat**	
Proximate	Average	Range	Average	Range	Average	Range
Energy (kcal)	62	59–66	99	71–118	66	58–74
Water (g)	87.8	87.3–88.1	83.2	82.3–84.0	87.7	86.4-89.0
Total protein (g)	3.3	3.2–3.4	4.0	2.7–4.6	3.4	2.9–3.8
Total fat (g)	3.3	3.1–3.3	7.5	5.3–9.0	3.9	3.3–4.5
Lactose (g)	4.7	4.5–5.1	4.4	3.2–4.9	4.4	4.2–4.5
Ash	0.7	0.7–0.7	0.8	0.7–0.8	0.8	0.8–0.8
Calcium (mg)	112	91–120	191	147–220	118	100–134
Phosphorous (mg)	91	84–95	185	102–293	100.4	90–111
Retinol (µg)	35	29–45	69	---	45	35–56
Vitamin E (mg)	0.08	0.07–0.08	0.19	0.19–2.0	0.05	0.03–0.07

Water is the main component in all milks, ranging from an average of 83% in Buffalo milk to 87.8% in cow milk [29]. Thus, Fresh milk contains 84–87% water in which all other constituents of milk are dissolved and in which are dispersed two different systems, namely fat globules enclosed within their protective membrane as an oil-in-water emulsion, and protein, containing

casein molecules and insoluble salts in a colloidal suspension. The main carbohydrate in milk is lactose, which is involved in the intestinal absorption of calcium, magnesium and phosphorus, and the utilization of vitamin D [30, 31]. Lactose also provides a ready source of energy for the neonate, providing 30% of the energy in bovine milk [32].

The mineral content of milk is composed of macro-elements (calcium, phosphorus, magnesium, sodium, potassium, and chloride) and numerous micro-elements present in trace quantities present in the inorganic part of the milk nutrients in various forms associated with different structural components. Conversely, vitamins are biologically active substances and together with other nutrients are the organic part of the milk nutrients, consisting of lipophilic vitamins (A, D, E, and K) and hydrophilic vitamins (B-complex vitamin and vitamin C). Thus, milk is a source of proteins, fats, sugars, vitamins, and major minerals [1, 6].

2.3.1 Mineral Composition of Milk

Milk has an important place in the human diet. It is almost a complete food for human beings having all the essential nutrients including minerals [33]. Minerals are important for growth, development and regulation of various vital functions in our body and approximately 4-6% of human body weight is composed of mineral elements [34]. These minerals are also important for building strong bones and teeth, and maintaining the ionic equilibrium of body fluids. Even in trace amounts, these minerals perform innumerable vital body functions. Milk contains more than 20 different minerals such as calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), phosphorus (P), zinc (Zn), iron (Fe), copper (Cu), manganese (Mn) etc. Milk is the most important source of bioavailable Ca and P in our diet, Ca through milk is better retained by the body in comparison to vegetables [33].

Mineral composition in milk is regarded as relatively constant, but the mineral content can vary mainly according to the lactation stage, the nutritional and health status of the animals [35]. Furthermore, reported data showed that the mineral contents of the various milk species and breeds varied considerably and their content appeared to be affected by genetic, physical and environmental factors [36]. In summary, milk is a food rich in precious minerals with mineral composition varying according to the animal species.

2.3.1.1 Selected Mineral Content of Milk

Calcium (Ca) is the main mineral found in milk, divided into the soluble and micellar phases. In the watery phase, Ca is present in its free form combined with citrate, inorganic phosphate and whey proteins. Two thirds of Ca is bound to casein in the micellar phase, related to organic phosphate (phosphoseryl residues of casein molecules) [37].

Partitioning of this mineral between these phases is complex, highly dynamic and influenced by different factors, such as temperature, ionic strength and pH. The Ca is thermodynamically equilibrated between the water and micellar phase, in relation to the chemical condition. Biological acidification processes such as lactose fermentation, during processing of dairy products, are primarily responsible for moving Ca into the water phase [38]. Ca bioavailability in milk is high [39] and certain milk components, e.g., lactose and phosphopeptide may improve the uptake of this mineral [40]. Milk represents an important source of Ca to the human diet. Phosphorus (P), an important component of milk and dairy products, is present in different forms of organic and inorganic phosphates and in various localizations. The organic form of P binds various molecules, mainly to casein molecules, in the micellar phase. The inorganic form of P is divided between the water and micellar phase. This mineral is transferred into the water phase mainly during acidification processes. P is mostly abundant in goat's and sheep's milk and is present in various animal foods [41]. Therefore, there is no shortage of this mineral for humans. Sodium (Na) and potassium (K) are present mainly as free ions in the water phase of milk and milk products and their concentrations are increased by salting [42].

2.3.2 Vitamin Composition of Milk

Milk is a good source of essential vitamins. Skimmed milk, but also some fermented milks, are often fortified with vitamin A. This is done to counteract the loss of vitamin A during the separation of the cream [43]. Vitamin C is degraded during pasteurization of the milk. Vitamins play an important role in intermediate metabolism as co-factors in many enzymatic reactions or in non-enzymatic physiologic functions, and do not function as structural components within the cell. The vitamin content in milk varies considerably among mammals and is closely related to the type of feeding given to the animals. In particular, increased vitamin E and carotenoids have been observed in milk and dairy products obtained from pasture fed cows compared with those obtained from milk of cows on corn silage feeds [44]. Milk and milk products contain both

water-soluble vitamins (vitamins B-complex and vitamin C), and liposoluble vitamins (A, D, E and K) [45]. The water-soluble vitamins are situated in the watery phase of milk and therefore present in skim milk; While the liposoluble vitamins are found in the lipidic phase, as in whole milk, cream and cheese. B complex vitamins perform numerous and important cellular functions, being implicated as cofactors in anabolic and catabolic reactions. Thiamine or vitamin B1 and riboflavin or vitamin B2, are produced from plants and micro-organisms [46].

In particular, riboflavin is produced especially from bacteria present in the rumen and is thus very present in the milk of ruminants. In general, both vitamin B1 and vitamin B2 are present in higher levels in ruminant animals compared with non-ruminant species. Niacin or vitamin B3 is primarily produced by microbial organisms of the rumen [46] and found in high concentrations, especially in sheep's milk. Pyridoxine or vitamin B6, synthesized from plants and micro-organisms, is involved in several biochemical pathways, such as in the metabolism of amino acids, lipids and gluconeogenesis [47]. The concentration of this vitamin is higher in sheep's and cow's milk than in milk of other animal species. Folic acid or vitamin B9 is a micronutrient involved in nucleic acid synthesis, is very sensitive to different physicochemical conditions [48] and is found in a higher content in cow's milk and human milk, compared to other species. Milk provides a significant source of folic acid to humans [49].

Cobalamin or vitamin B12 originates from microorganisms in the digestive tract, through microbial synthesis in the rumen [50, 51]. It is involved in various important functions of the organism and in milk is mainly bound to proteins. Vitamin B12 is mostly present in ruminant milk, which contributes largely to human intake. In particular, the vitamin is found in higher concentration in cow and sheep's milk. Ascorbic acid or vitamin C has been known as an antioxidant molecule [52], found in milk but not in milk derivatives, as a result of its degradation during their processing. Vitamin C has a high sensitivity to light and heat treatments and milk cannot be considered an interesting source.

The fat-soluble vitamin content of milk is related to the overall fat content of milk. Vitamin A is found in various forms: retinol, retinal and retinoic acid and is highly sensitive to oxidation, light or different oxidant factors [53]. The concentration in milk declines after heat-treating and

acidification processes [54]. The content of vitamin A is higher in breast and sheep's milk compared to other animals.

2.3.3 Protein Composition of Milk

The proteins of milk are divided into caseins and whey proteins. Caseins include αs1-casein, αs2-casein, β-casein, and κ-casein and make up 80% of the total protein content. The other 20% consists of whey proteins, including β-lactoglobulin, α-lactalbumin, serum albumin and immunoglobulins. The distinction between the two groups is based on solubility at pH 4.6 [55].

The casein fraction is organized in micelles, a large network with a hydrophobic core and hydrophilic outer layer allowing the caseins to remain suspended in the aqueous phase. Caseins are small proteins, form little tertiary structure and form hydrophobic bonds with each other which make them relatively heat stable. Clusters of calcium (Ca) and phosphate (P) form as milk is synthesized, and due to their low solubility the caseins rapidly bind CaP to prevent nucleation. K-casein differs from the other caseins with its ability to become glycosylated with oligosaccharides consisting of galactose, and one or two N-acetyl neuraminic acid (NANA) residues. These groups have negative charges, and are thus hydrophilic. The κ-casein works as a chain terminator in the 3D structure of the casein micelle. The peptide bond in the glucomacropeptide is readily hydrolyzed in presence of enzymes such as rennet, and this property is used in the cheese making process [56].

Whey proteins build a heterogeneous fraction with the common feature that they are soluble in the serum phase of milk. They are typically globular proteins and the two major ones are β-lactoglobulin and α-lactalbumin, which are both synthesized by the secretory cells. β-lactoglobulin makes up the major part of the whey proteins, α-lactalbumin, as previously mentioned, is a coenzyme in the synthesis of lactose. Other major whey proteins include the immunoglobulins, and IgG, IgA and IgM occur at high levels in colostrum with the biological function to provide protection to the newly born calf [56].

Protein-mineral interactions in milk are essential for the functionality of milk and the nutritional benefits that it provides. The protein or peptides of milk can function as carriers, chelators of various minerals and thus enhance or inhibit bioavailability [57]. Milk and milk products provide

a wide range of bioactive compounds, among them proteins such as caseins, whey proteins, and other minor constituents, have been seen to exhibit important biochemical and physiological functions on human metabolism and health [58–60]. The caseins, which accounts for about 80%, are divided in α-caseins, which in turn comprise s1-casein, s2-casein, β-casein, and κ-casein. The whey proteins are a heterogeneous group of heat labile globular protein constituted mainly by α-lactalbumin, β-lactoglobulin, serum albumin and immunoglobulins, and to a lesser extent by lactoferrin and lysozyme [61, 62]. In particular, lysozyme is a peptide with bactericidal action, since it breaks the bacterial cell wall. In the whey protein fraction, there are also enzymes, hormones, nutrient transporters, growth factors, disease resistance factors, and others.

Bioactive peptides have been defined as specific protein fragments that have a positive influence on physiological and metabolic functions or condition of the body and may have ultimate beneficial effects on human health [63]. Bioactive peptides are released during gastrointestinal digestion and fermentation of food materials by lactic acid bacteria. The caseins, αs1-, αs2-, β- and k-casein, are most often reported as precursors of peptides containing binding sites, phosphoseryl and carboxyl, for different minerals [64]. Indeed, caseinophosphopeptides has the function of carriers for different minerals by forming soluble organophosphate salts, especially Ca^{2+} ion. Lactoferrin is among the whey proteins most involved in forming mineral bonds; In fact, it is an iron-chelating glycoprotein, which plays an important role in iron absorption in the intestine [65].

2.3.4 Carbohydrate Composition of Milk

Lactose (milk sugar) is the principal carbohydrate and a major component in milk. It is a naturally occurring sugar that is almost exclusively found in mammalian milk [66, 67]. Lactose differs from other sugars, such as sucrose, in functional properties because it has a low relative sweetness. It is about 20% as sweet as sucrose. Lactose is made up from the building blocks galactose and glucose unit [68], connected by a glycosidic linkage in β-configuration, described as β-D-galactopyranosyl-(1→4)-D-glucopyranose (Figure 2.1).

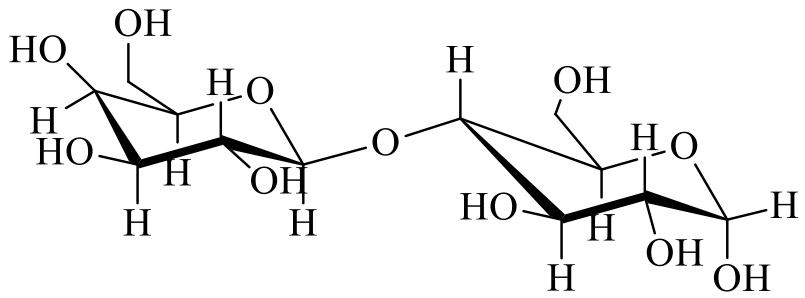

Figure 2.1: The structure of lactose

Lactose is a reducing sugar [68] unique to milk, and it has been found in milk from nearly all mammals. For lactose to be digested, it must be broken down in the intestine by the enzyme lactase to its component monosaccharide's glucose and galactose. Glucose can then supply energy to the young animal. Many people are unable to consume cow's milk and dairy products because they are unable to digest lactose after weaning.

Most infants possess the enzyme lactase and can therefore digest lactose, but this ability is lost in many people after weaning (commonly after the age of two). Lactose provides 30% of the energy from milk [69], and gives milk its sweet taste, although lactose has low relative sweetness.

2.3.5 Fat Composition of Milk

The principal fat in milk is a complex combination of lipids called triglycerols (esters of three fatty acids with one molecule of glycerol). Nearly all lipids in milk are found within the milk fat globules (MFG). Milk fat globules are spherical colloidal assemblies of milk lipids with a core rich in triacylglycerol. Triacylglycerol make up 98% of the milk fat, but also small amounts of di- and monoacylglycerol, cholesterol and cholesterol esters, free fatty acids, and phospholipids are present. The fat composition varies with species, breed, feed, stage of lactation, number of lactations, etc. The milk fat is enclosed by a membrane (MFGM) built up by phospholipids, proteins and glycoproteins which prevent aggregation and coalescence. The size of the MFG varies from 0.1 - 20 μm [70]. Milk fat contains approximately 400 different fatty acids. Fatty acids are described as saturated or unsaturated depending on the amount of hydrogen in the carbon chain of the molecule; milk contains both saturated and unsaturated fatty acids. Unsaturated fatty acids may be further classified as monounsaturated or polyunsaturated (depending on the number of double bonds in the carbon chain of the fatty acid molecule).

Again, milk contains fatty acids from both groups but most of the fat in whole cow's milk (around 65% or approximately 70%) is the saturated type [71]. Polyunsaturated fats include fatty acids called the omega-6 and omega-3 fatty acids (these names refer to the position of the double bond in the carbon chain of the fatty acid molecule). Milk contains the omega-6 essential fatty acid linoleic acid and the omega-3 fatty acid linolenic acid. These are called essential fatty acids because they are essential to health but cannot be made within the body and so must be obtained from the diet [72].

2.4 Composition of Cow's Milk

Milk can be considered a source of macro- and micronutrients, together with bioactive substances, and also contains a number of active compounds that play a significant role in both nutrition and health protection [73]. Cow is the most popular dairy producer animal in the world, providing essential nutrients and an important source of dietary energy, high quality protein and fat [29]. Milk composition of cows varies depending on breed, the animal feed, stage of lactation, genetics, physical and environmental factors [28, 74].

Cow's milk contains some major elements such as calcium, phosphorus and magnesium, in addition to potassium, and sodium and a wide range of trace elements including zinc, copper, iron, manganese and iodine. Thus, cow's milk is an important source of protein, minerals and vitamins in the human diet. Cow milk accounted for 83% of global milk production in 2010 [23]. Cow milk generally contains between 3 and 4 g of fat/100 g, although values as high as 5.5 g/100 g have been reported in raw milk. Most milk consumed now contains a standardized fat content of around 3.5 g/100 g. Milk chemical composition is an important parameter, which indicates its nutritional qualities. Milk having high total solids has high fat, protein, and casein content [22].

2.5 Milk Classifications

Milk can be classified according to its fat content, for example as whole milk, skimmed milk, semi-skimmed milk, low-fat milk and standardized milk. It can also be classified according to the processing procedures it has undergone, such as pasteurized milk, sterilized milk, and extended shelf-life (ESL) milk and ultra-high-temperature (UHT)-treated milk, among others. The FAOSTAT definitions for various milk and milk products are given below in italics, where

available. The FAOSTAT codes are given within brackets. CODEX definitions are given only where FAOSTAT definitions are not available or where additional information is needed.

2.6 Food Pasteurization

Food pasteurization is a process that involves heating food to a specific temperature for a certain period of time to eliminate harmful microorganisms such as bacteria, viruses, and parasites that can cause illness. This process was first introduced in the late 19th century by French microbiologist Louis Pasteur, and it has since become a widely accepted practice in the food industry. The primary purpose of pasteurization is to reduce the risk of foodborne illnesses caused by microorganisms present in the food. Some of the common food products that are pasteurized include milk, juices, eggs, cheese, and other dairy products. The process is also used for canned foods, such as soups and vegetables, to ensure that they are safe for consumption and have a longer shelf life [75].

Milk has a flourishing population of microbes. This is vital for natural coagulation of milk, but it can be harmful that is why various methods are used to pasteurize or sterilize the milk, thus avoiding deterioration and prolonging the length of time if it can be stored.

Thus, milk pasteurization is a method of exposing milk to elevated temperatures for a period of time as a means of reducing the bacterial contamination. This process kills bacteria that can cause diseases in humans and animals. It is important to note that pasteurization is not sterilization. Pasteurized milk still may contain measurable amounts of bacteria. Pasteurizing poor quality milk with a very high concentration of bacteria may allow some viable pathogenic bacteria to survive the pasteurization process.

2.6.1 Types of Pasteurization

There are two common methods of pasteurizing milk: batch pasteurization and continuous flow high temperature, short-time (HTST) pasteurization and/or ultra-high-temperature (UHT). Standard batch pasteurization is accomplished when a batch (usually a vat or tank) of milk is heated to 145 °F (63 °C) for 30 minutes. Thereafter, the milk is cooled and can be ready for consumption. Batch pasteurizers should be equipped with an agitator to allow for even heating. There are concerns about the volume of milk to be heated and the time to do it. Very large batches take several hours to reach the desired temperature and there are concerns that some

bacteria may become heat resistant, surviving the pasteurization process. The cleaning process of these units is most often done manually.

The process of HTST and UHT is different. Milk is circulated through a network of heated coils, rapidly heated to 161 °F (72 °C) and held there for 15 seconds, while UHT pasteurization involves heating the food product to a much higher temperature of at least 275 °F (135 °C) for a much shorter period of time, typically less than one second. These types of systems are also equipped to automatically cool the milk quickly to feeding or storage temperature. Continuous flow pasteurization is much more rapid than batch pasteurization and offers more opportunities for energy conservation. Continuous flow systems are generally more difficult to clean, requiring a cleaning procedure similar to that used in milking systems, but in many cases the cleaning process can be automated.

HTST pasteurization is commonly used for milk and other dairy products, as it does not significantly affect the taste or nutritional content of the product. However, it may not be effective in eliminating all microorganisms, and there is a risk of recontamination after the pasteurization process.

On the other hand, UHT pasteurization is more effective in eliminating microorganisms and has a longer shelf life, but it may affect the taste and nutritional content of the product [76]. In addition to the HTST and UHT methods, there are other pasteurization methods that are used for specific types of food products. For example, the low temperature long-time (LTLT) method is used for certain types of cheese, and the batch pasteurization method is used for egg products. Despite its benefits, there are some concerns about the use of pasteurization in the food industry. Some argue that the process can destroy beneficial nutrients and enzymes in the food, which can have negative effects on human health. However, the evidence for this claim is not conclusive, and the benefits of pasteurization in reducing the risk of foodborne illnesses far outweigh any potential negative effects [77].

2.6.2 Effects of Thermal Treatments on Milk Micronutrients

Drinking milk is produced from raw milk through a series of steps that include: collection, normalization that allows reaching the right fat content based on the type of milk to be produced (skimmed, partially skimmed or whole), homogenization to break up the fat globules suspended

in the milk to distribute them throughout the entire volume avoiding the accumulation of fat on the surface, heat treatments such as pasteurization or ultra-high temperature (UHT) sterilization and finally spillage into commercial packaging for storage and sale [78].

It is well known that heat treatments of milk affect both mineral distribution (especially calcium and phosphorus) between its aqueous and colloidal phase, e.g., a reduction in the solubility of calcium phosphate is observed [31,79], and on the concentrations of micronutrients in milk. While no substantial loss of B complex vitamins as vitamin B2, B3, B5, B6 and B7 by heat treatment was observed (only a reduction of less than 10%) [80], losses of vitamin B1, B12 and C increased from 10–20% in pasteurization and UHT treatment to 90% loss of B12 in bottle sterilization and evaporation [80,81]. The fat-soluble vitamins of milk are not much affected by heat treatment. Indeed, no significant differences in vitamin A and carotene content in raw, pasteurized and boiled milk were observed [82]. Since Vitamin A is susceptible to light exposure, the choice of packaging material is a critical parameter affecting vitamin stability in heat-treated milk, which shows major stability in milk stored in dark bottles and transparent polyethylene (PET) bottles [83]. Similar to vitamin A, vitamins D, E and K are more susceptible to light exposure compared to heat treatment (especially vitamin D) [31,84].

Overall, current industrial practices for heat treatment of milk result in limited decomposition of milk vitamins as heat-triggered interactions of vitamins with milk components (i.e., proteins, fat) may well play a protective role on their heat-stability [85].

The milk processing objective is to reduce microbial pathogens in raw milk, but several multiple evidences showed that homogenization and pasteurization, through disruption of fat globules and casein micelles, alter the milk fat and protein molecular structure, increasing the allergenicity of processed milk [86]. The casein, betalactoglobulin and alpha-lactalbumin are mainly involved in cow's milk protein allergy. The exclusion of milk and derivatives from the diet is known to cause nutritional deficiencies in macro and micronutrients [87, 88]. However, not excluding these products from the diet in people allergic to proteins or also lactose intolerant (due to the deficiency of the lactase enzyme), produces a wide spectrum of adverse symptoms that mainly affect the gastrointestinal tract but also causes dermatitis and respiratory problems [89, 90].

2.7 Milk Processing Practices in Ethiopia

In Ethiopia, milk and milk products are mainly used for home consumption as they have high nutritional value. In addition, it is a source of cash income to purchase farm inputs like feed, fertilizer and improved crop varieties as well as food and non-food items like educational materials for their children [91]. However, the quality of milk produced in Ethiopia is poor and below the standard. This is due to poor pre-milking and post-harvest handling practices and highly perishable characteristics of the milk [92]. Thus, milk processing is a crucial measure for the preservation of food constituents as sources of nutrients and cash for several people within the world [93]. In Ethiopia, milk processing is usually traditional fermented milk making, with no defined starter culture, which is mostly made of cow's milk. This can be due to several reasons including high ambient temperatures, small daily quantities of milk, consumer preference and increased keeping quality of sour milk [94]. Milk products are more stable than fresh milk because they are more acidic and/or contain less moisture. Among the standard fermented milk products, butter, Ayib (Ethiopian cottage cheese), and Ergo (Ethiopian fermented milk) represent the foremost marketed products next to milk. It's therefore important to appear into their processes about hygienic conditions practiced during handling [95]. Milk processing is typically designed to get rid of water from milk or reduce the moisture content of the milk products. Generally, milk processing isn't well developed in Ethiopia [96].

2.7.1 Traditional Milk Processing, Preserving and Cleaning

Farmer's process milk produced in their farms to other milk products in order to increase its shelf life. Out of the total milk produced was used for traditional processing and converted it into dairy products. The major products of the traditional milk processing were naturally fermented milk, traditional butter, buttermilk, cottage cheese, whey and ghee [97]. The processing of butter-milk into cottage cheese of peri-urban producers was slightly higher than that of urban producers. The urban farmers more frequently used butter-milk for bucket-feeding of calves.

The urban producers do not process butter-milk into cottage cheese and whey due to the lacking availability of firewood for cooking. The majority of dairy farmers who live far from urban centers in Ethiopia processed milk into different byproducts [98]. Converting; milk to other products may be different from area to area depending on: environmental temperature, on quality and disease condition of animals [97].

The milk producers used different techniques to preserve fresh milk without clotting, such as smoking of the container and boiling of fresh milk before collection, or refrigeration. In different production systems, smoking was the predominant practice. Most urban farmers use a refrigerator, an option which was almost not present in the peri-urban areas.

Cooling by putting the container with milk into a cold-water bath was practiced by the peri-urban producers [98]. It is obvious therefore, that for technical and economic reasons technologies in fluid milk processing such as steam-pasteurization, sterilization and aseptic packing are not common on Ethiopian smallholder farms [99].

Smoking of milking and storage containers was done by using Kosorote (Ocimumhaardiense), Tejsar (Cymbopogan martini), Tenadem (Rutachalepensis) and wood splinters of 'Weyira' (Oleaafricana) were used [98]. Smoking is used to develop desirable flavor and aroma [100], increase shelf life of milk and facilitate fermentation. In addition to imparting pleasant flavor, it facilitates fermentation and increases shelf life of milk and milk products. Smoking has antimicrobial activity, thus inhibiting the growth of microorganisms in milk. The purpose of smoking, the practice of smoking the vessel by burning wooden chips of specific trees and shrubs, is to improve the taste and flavor of milk products, to reduce bad microorganisms and to increase the shelf life of the products [101], and also smoking plants such as Cheba (Acacia nilotica), Abalo (Combretummolle), Ader (Dichrostachyscinerea), Asta, Kega (Rosa abissinica) and Woira (Olea Africana) [102-105] respectively for better flavor and aroma of milk and milk products [100,106].

2.7.2 Modern Milk Processing Industries in Ethiopia

Currently, in Ethiopia there are thirty-two milk processing industries established in different parts of the country. The major dairy processing plant products are pasteurized milk, yogurt and different kind of cheese (Provolone, Mozzarella, Gouda and Feta). About 83.4% of the total milk produce was pasteurized milk and about 12.69% was yogurt [107].

2.7.2.1 Major Private Dairy Enterprises

Commercial processors are those adopting modern technology with the majority of their output being pasteurized milk in packs of 500 mL. There are over 22 medium- and large-scale dairy processing companies currently in Ethiopia with nine of them operating in Addis Ababa and the

rest in other major regional cities (Appendex I). The major types of inputs used for milk processing industries are raw milk, plastic pouches, yogurt cups, rennet and cultures (for yogurt and cheese making). Dairy industries obtained raw milk directly from producers and indirectly from collectors, retailers and milk suppliers [107]. Dairy processing industries, which are found in Ethiopia produce up to 24 dairy products and among them pasteurized milk, skimmed milk, yoghurt, fermented milk, table and cooking butter, cheese, cream and ice cream. The milk processing industries found in Ethiopia process the milk into pasteurized milk about 83.4% of the total milk produced in industries and 12.69% covered by yogurt. Average production of pasteurized milk in dairy plant was 8740.48 ± 16239.63 liter/day, yogurt 1330 ± 2280.46 liter/day and Gouda cheese was 33.81 ± 83.52 kg per day [107].

2.7.2.2 Handling, Collection, Bulking, and Transportation of Milk

Milk is also highly perishable and can easily be adulterated whilst the quality of the milk is highly dependent on farm management. Equipment used for milking, processing and storage determine the quality of milk and milk products. The use of plastic and traditional containers can be a potential source for the contamination of milk by bacteria, because this allows the multiplication of bacteria on milk to contact surfaces during the interval between milking [108, 109].

Type of materials used for milking and methods employed in cleaning practices were common in the study with minor differences. Smoking of milk utensils prior to milking and churning is a common traditional practice in most parts of the country [110]. Those farmers that could not practice washing the udder of milking cows witnessed that the calf suckles the udder of the cow before milking, thus there is no need for washing. A good supply of clean water is essential for the production of quality milk. The utensil used for milking should be clean using drinking water. Unsafe water is a major contributor to poor quality of milk.

Milk processing industries opened Milk collection points from smallholder producers around Addis Ababa to feed the processing plant. Raw milk collection was further strengthened and expanded to a seven kilometre radius from the city. This arrangement attracted a substantial number of smallholder farmers that produced and delivered small amounts of milk from their indigenous cows. For example, Lame (Sholla), is now a private company, operating with 25

collection centers located around Addis Ababa, 13 of the centers are near Selale, five close to Holetta, and seven around Debre Birhan. At the collection points, milk is subjected to a field acidity (alcohol) test for freshness and a lactometer reading for possible adulteration (addition of water) and removal of cream. The accepted milk is transported to the nearest chilling center, where it is cooled to temperatures below six degrees Centigrade. Milk is usually delivered to the collection centers and milk cooperatives by producers either on foot or donkey back.

Producers sell the surplus milk produced to their neighbors and/or in the local markets, either as liquid milk or in the form of butter and/or Ayib [111]. This system is characterized by no license to operate, low cost of operation, high producer prices as compared with the formal market and no regulation of operation [112]. The hygienic condition of milk and milk products channeled through this system is also poor. This is mainly due to the prevailing situation where producers have limited knowledge of dairy product handling coupled with the inadequacy of dairy infrastructure such as cooling facilities and unavailability of clean water in the production areas. In the formal system, milk is collected at the cooperative or private milk collection centers and transported to processing plants. In this system, milk quality tests (principally acidity using alcohol and clot-on-boiling test, and density) are performed on delivery, thereby assuring the quality of milk.

This has encouraged the producers to improve the hygiene conditions, storage and transportation of the milk in order to avoid rejection of the product on delivery to the collection center.

In the informal system, milk is distributed from producers to consumers (neighbors and/or in local markets) and milk products mainly in local markets. In the formal system milk is distributed by milk cooperatives and unions and the private sector. Milk collected at milk collection centers is supplied directly to consumers in the urban towns and the surplus is collected by large dairy enterprises such as Lame (Sholla), Sebeta Agro Industry (Mama) and Family Milk and transported by bulk tankers to the respective processing plants. These dairy enterprises process and pack the fresh milk collected for distribution to consumers in urban areas through agents and retailers. Homogenized, pasteurized and standardized (2.7–2.8% milk fat) milk packaged in half-liter capacity plastic packets are distributed.

2.7.2.3 Pasteurized Milk Quality

Despite milk being a highly nutritious food, it can be easily contaminated with physical, chemical and microbiological hazards. Milk quality refers to a blend of characteristics such as chemical, physical, bacteriological and aesthetic that boosts up the acceptability of the milk and milk products. Safe milk should be free from pathogenic organisms and other contaminants that may constitute health hazards [113]. Specific gravity, chemical composition and microbial quality of milk are important to determine milk quality and safety. While for milk products, microbial quality and chemical composition are determinant for its quality and safety [113].

2.8 Toxic trace Elements in Milk

Milk is an important source of protein, minerals and vitamins in the human diet. It contains some major elements such as calcium, phosphorus and magnesium, in addition to potassium, and sodium and a wide range of trace elements including zinc, copper, iron, manganese, iodine, and selenium. These are essential micro-nutrients and have a variety of biochemical functions in all living organisms. Some of them form an integral part of several enzymes. However, contamination of milk and dairy products by toxic metals can be a possible health risk to human population.

Milk can be contaminated by toxic elements, the most important of which is the element lead (Pb), which is known to have deleterious effects on the developing nervous system of children [114, 115]. Furthermore, Milk can also be contaminated by other toxic metals such as cadmium (Cd), mercury (Hg), arsenic (As) and nickel (Ni) and even by high concentrations of essential elements such as Co, Cr, Cu, Fe and Zn [116,117]. Moreover, the essential trace element profile of milk, particularly toxic element residues, is largely affected by the environment where the cows are raised [115, 117,118].

Heavy metals mainly enter cow's milk through cattle feed and drinking water (as well as via the atmosphere). The feed and water can, in turn, be contaminated through the soil via sewage sludge used as fertilizer, artificial fertilizers, metals used in fungicidal agents and other agricultural chemicals, and also via wastewater from various industries. The risk of milk becoming contaminated is particularly high in areas affected by anthropogenic pollution, such as smelting or mining areas and highly industrialized regions, allowing the transfer of metal

contamination to the atmosphere, soil, water, animal feed, animals and their products, and finally to humans [114,115,117,119–121]. Thus, the world-wide contamination of milk with undesirable substances via animal feeds, heavy metals, mycotoxins, dioxins and similar pollutants is considered to be of great concern to public health because milk is widely consumed, especially by children [115] and due to their toxic effects on humans and wildlife. In the case of Pb, milk can become contaminated when cows graze and drink water at roadsides. In addition, factors related to the manufacturing practices (particularly hygiene during milking) and possible contamination from the equipment during processing [116, 117] can also increase the concentration of this toxic element in milk. Hence, the presence of toxic heavy metals in milk reduces its nutritional value and poses a hazard to human health. The presence of toxic metals in the food chain is the result of environmental pollution and their concentrations need to be controlled constantly because Milk and dairy products are staple components of a daily diet of contemporary consumers, especially children. Thus, it is crucial to regularly monitor milk quality, paying special attention to toxic metals. Their concentration in milk, especially in industrial regions, may serve as a direct bioindicator of the quality of milk and its products, but can also be an indirect indicator of contamination in the environment where milk is produced [122, 123].

2.9 Proximate Analysis of Milk

Proximate analysis is a type of chemical analysis used to determine the nutritional value of food and feed materials. It involves the identification and quantification of the major components in a sample, such as moisture, ash, protein, fat, and carbohydrates. These components are called proximate because they provide a basic understanding of the composition of the sample, which can be used to predict its nutritional value. Proximate analysis is widely used in the food industry to evaluate the quality and safety of food products. It can help to determine the nutrient content of foods, identify potential contaminants, and assess the shelf life of products. The proximate analysis process involves a series of laboratory tests that are designed to isolate and quantify the major components in a sample. These tests may include measuring the amount of moisture in a sample, burning the sample to determine the ash content, extracting the fat and protein content with solvents, and determining the amount of carbohydrates present. Overall, proximate analysis

provides important information about the nutritional composition of foods and feeds, which is essential for ensuring that people and animals receive the proper nutrients for good health.

Moisture analysis is the first step in proximate analysis, as it determines the amount or percentage of water in the sample. “The moisture content of any food is a measure of its water activity and may be used to define its stability and susceptibility to microbial infection [124, 125]. This is followed by ash analysis, which determines the amount of inorganic matter in the sample. The ash is the non-gaseous and non-volatile residue that is left behind after the thorough incineration of any matter. The ash is home to minerals. The quantity of mineral content is directly proportional to the content of Ash present in the food materials [126]. Crude protein analysis determines the total protein content of the sample. Proteins are made up of amino acids. The crude fat analysis measures the number of lipids in the sample. The primary function of Dietary fats is to increase the palatability of food by absorbing and retaining flavors. Fibers are the indigestible part of the diet, which is also called bulk or roughage. Fibers support good health and are known to lower cholesterol levels [127]. Heart disease, rectum and colon cancer, varicose veins, diabetes, phlebitis, appendicitis, obesity, and even constipation have all been linked to low-fiber diets [128, 129].

2.9.1 Moisture Content

Moisture content of food is of great importance to every food processor as a number of biochemical reactions and physiological changes in food depend very much on the moisture content. Furthermore, moisture content has an effect on the stability and quality of foods. Therefore, moisture determination is one of the vital components of food evaluated in the laboratories. Several methods are available for the determination of moisture in foods. Some of these methods are indirect distillation (or drying methods), direct distillation methods, use of electrical moisture meters and chemical methods. The most commonly used method however is the indirect distillation method employing drying ovens [130]. The moisture analysis was carried out immediately after the samples were mixed well and taken out of the plastic bags.

2.9.2 Titratable Acidity (TTA) and pH

The titratable acidity is an expression of percentage lactic acid [131], which may be determined by titration of a known amount of milk sample with 0.1N NaOH using phenolphthalein as an

indicator. Measuring this parameter is very important for two reasons: first, it's a method of storage, and second, it determines the taste of the fermented milk [132].

2.9.3 pH

pH is the term indicating the positively charged hydrogen atom concentration, and it is a measure of the solution's acidity. pH plays an important role in determining the standard of fermented milk since it indicates the amount of acid present within the milk [133]. Moreover, in line with [133], lactic strains can ferment lactose into a carboxylic acid, with a rise in acidity and a decrease in pH of fermented milk.

2.9.4 Total Solids (Dry Matter Content) and Solid–not-fat (SNF) Content

The total solids content is a measure of the amount of material remaining after all the water has been evaporated. Total solids content of milk samples were determined using the Gravimetric Method procedure given by Bureau of Indian Standards BIS [134].

Solid–not–fat (SNF) portion consists of protein (primarily casein and lactalbumin), carbohydrates (primarily lactose), and minerals (including calcium and phosphorus). Solids-not-fat consists of all solids in milk other than fat. Milk with high solids–not–fat is valuable to the consumer for its flavor and nutritional value and to the manufacturer of milk products, especially relating to cheese yield. Solid–not–fat (SNF) is the total solids content minus the fat content.

2.9.5 Total Ash Content

Ash content in foodstuffs indicates the presence of inorganic minerals in these foodstuffs. Thus, ash level is a reflection of mineral contents in a sample [135]. In general, the mineral elements in food stuff are often small (less than 1% of the food). The individual elements tend to vary depending on the particular element and on type of food. According to [136], mineral elements are classified on the following basis: (I) Major Elements (> 0.01% or 100 ppm) Calcium, Phosphorus, Chlorine, Sulphur, Magnesium, and sodium; (II) Trace elements (< 0.01% or 100 pm) Arsenic, Copper, Iron, Nickel, Tin, Chromium, Fluorine, Maganese, Selenuim, Vanaduim, Cobalt, Iodine, Molybedenum, Silicon and Zinc; (III) Non-essential toxic mineral elements Beryllium, Lead, Cadmium, palladium, mercury and Thallium.

Ash in food constitutes the residue remaining after all the moisture has been removed as well as the organic materials (fats, proteins, carbohydrates, vitamins, organic acids etc.) have been burnt

away by igniting in a muffle furnace at a temperature of around 550 °C. This result in the oxidation of organic constituents to volatile materials considered as carbon dioxide, nitrogen oxides and sulphur dioxide. The total ash content was determined according to [137].

2.9.6 Protein Content

Protein has a large function in the human body (For example, building the structural organs) and in fact, the human body is about 45% proteins. It is an essential macromolecule without which our bodies would be unable to repair, regulate or protect it. Essential body processes such as water balancing; nutrient transport and muscle contractions require protein to function [138]. Protein also aids in the formation of antibodies that enable the body to fight infections. Protein serves as a major energy supplier [139]. The building blocks of protein are Amino acids. Proteins are therefore polymers of amino acids, most of which are α-amino acids, having the general formula $NH_2CHRCOOH$. It is the only macronutrients in foods that contain nitrogen. The nitrogen in protein becomes the basis of the estimation of protein in food. There are many methods of protein determination. Some of which include; Kjeldah method, direct distillation method. Dye– binding methods, formaldehyde titration and spectroscopic methods. The formaldehyde titration method was used in the present work [23, 140].

2.9.7 Total Fat Content

Raw milk is usually composed of about 4% fat, 3.2% proteins, 4.6% lactose and a number of micronutrients [141]. However, the actual composition of bovine milk is influenced by the breed, lactation stage, as well as feeding practices. The vast majority of the total fat is contained in the form of fat globules. They are synthesized and secreted in the mammary gland. Milk fat is found forming globules surrounded by a membrane of lipoprotein nature composed mainly of phospholipids and glycoproteins and whose nucleus consists mainly (95%) of triglycerides (TAG), and has a hydrophobic character [142]. But in its composition we can also find other simple lipids such as monoglycerides, diglycerides and cholesterol esters, and some more complex ones such as tocopherols [143]. Thus, for extraction of fat from the milk sample (oil in water emulsion), fat globules are to be broken and the milk proteins, which form the membrane around the globules of fat, can be coagulated with alcohol.

The free fat is then dissolved in acid (conc. HCl) or alkali (conc. Ammonia). Then, the separated, fat is then extracted with the help of organic solvents such as petroleum ether, diethyl ether, chloroform, methanol, ethanol, isopropanol, n-butanol, acetone, acetonitrile, isopropyl ether, dioxane, tetrahydrofuran, dichloromethane, pentane, hexane, benzene, cyclohexane, iso-octanol, or mixtures of these solvents.

Fat content determination in milk and its products can be done by various methods, which are based on two principles: (a) Centrifugal Separation of Fat and (b) Extraction of Fat with Organic Solvents. The most commonly used being the Roese–Gottlieb [144], Gerber [145], Babcock [146], Rapid detergent method [147] and Mojonnier [148]. The determination of fat content gravimetrically by addition of ammonia and alcohol to a known amount of the milk and then extraction of the fatty substances with a solvent followed by evaporation of the solvent and weighing of the residue is known as the Rose Gottlieb Method (Reference Method), which was used in the present work [144].

2.9.8 Carbohydrate/Lactose Content

Percent lactose was determined by subtracting the fat, protein and total ash percentages from the total solids [23, 149].

Percent lactose = percent total solids – (% fat + %protein + % total ash)

2.10 Mineral Analysis of Milk

Minerals are critical building blocks of bones, teeth, tissues, muscles, blood, and nerve cells and are crucial for the total development of both mental and physical beings. Calcium provides animal bone with rigidity and support, and deficiency of calcium causes tetany [150]. Potassium aids in the control of electrolytes, water, acid-base balance, and muscular function in the body [151]. In addition, potassium interacts with macro ions to activate some enzymatic activities and interacts with proteins and nucleic acids. The regulation of plasma volume, acid-base balance, neuron function, and muscle contraction are all fundamentally influenced by sodium [152, 153].

Mineral contents were determined by atomic absorption spectrometry [154]. Mineral analysis in biological samples, especially in milk and milk products, are important because of their vital role in various biological processes. Thus, the determination of minerals such as Ca, Mg, Cu, Mn, Ni,

Co, Cr and Pd in pasteurized cow milk samples were carried out using atomic absorption spectrophotometry ((BUCK SCIENTIFIC MODEL 210VGP, equipped with deuterium arc background correctors) after acid digestion of the samples. In flame atomic absorption spectrophotometry, the concentration of each element was calculated by reference to a standard curve prepared using standard stock solution of each element.

3 MATERIALS AND METHODS

3.1 Sampling and Sample Collection

Pasteurized Milk samples for the study were collected randomly from retail shops in Addis Ababa, Ethiopia during the period 2–3 February 2024. Seven pasteurized cow milk sample (500 mL milk packaged plastic) from each brand of milk were collected from different retail shops in Addis Ababa, Ethiopia. Totally, twenty–one pasteurized cow milk samples from seven different brands of pasteurized cow milk available in local markets in Addis Ababa, Ethiopia were collected and transported in a cooled box with an ice pack into the Hawassa University laboratory and Chilled at 4 °C before immediate analysis. The chemical composition of the pasteurized milk samples according to the package label is shown in Table 3.1.

Table 3.1: Proximate values of the selected milk sample as claimed by the producers

Sample name or sample code	**Carbohydrate Lactose (%)**	**Fat (%)**	**Protein (%)**	**Expire Date: dd/mm/yy**
Mama (AA/B1)	3.72	2.7	3.07	08/02/24
Loni (AA/B2)	4	2.7	3.07	07/02/24
Zagol (AA/B3)	4.2	2.8	3.07	09/02/24
Harme (AA/B4)	4.6	2.7	3.5	11/02/24
Emit (AA/B5)	4.6	2.8	3.5	09/02/24
Shola (AA/B6)	4.7	2.7	3.07	09/0224
Addey (AA/B7)	4.8	2.8	3.0	10/02/24

3.2 Preparation of Sample for Mineral Analysis

Many researchers use microwave digestion techniques to solubilize biological samples [155, 156], however, without such costly equipment available, open acid digestion presents the most viable option. The digestion method used in the present study was an open acid digestion by means of nitric acid (HNO_3, 70% (w/v)) and perchloric acid ($HClO_4$, 70% (w/v)) [157]. The acids were used to release all metals bound in the milk sample into the solution and then individual determination of the metals by flame atomic absorption spectrometry (FAAS) were possible. The acid digestion method used in this study was optimized for variables such as combination of digestion acids, digestion time and digestion temperature.

3.2.1 Optimization of digestion Procedure

Digestion of food substances involves heating the sample and an acid or a mixture of the mineral acids on a heating mantle to the boiling point of the mixture to get a clear solution. Thus, the basic requirement for sample preparation for metal analysis is to get an optimum condition for the acid digestion of samples. However, there exists no single open acid digestion procedure for the analysis of metals for all biological materials [158]. The nature of the biological sample, the analyte, the reagent availability and equipment usually play a decisive role in the selection of the digestion procedure because it helps to select the best conditions suitable to give the highest yield of extractable metals. Thus, the optimum condition to prepare pasteurized milk samples for metal analysis were evaluated by varying parameters such as volume of acid mixtures used, volume of sample used, digestion time, and digestion temperature. The optimum conditions were selected based on minimum reagent volume consumption, minimum digestion time, and clear digestate solution.

3.2.2 Optimized Acid digestion Procedure for Mineral Analysis

Applying the optimized conditions (i.e., 5.0 mL HNO_3 (70%): 3.0 mL $HClO_4$ (70%) volume ratio of reagents, 200 °C digestion temperature and 2:00 hours digestion time) for the digestion of 5.0 mL pasteurized milk samples, 5.0 mL of pasteurized milk samples was transferred into a 250 mL round bottom flask, and a mixture of HNO_3 (70%) and $HClO_4$ (70%) with a volume ratio of 5:3 (v/v) was added and the mixture was digested on a Kjeldahl digestion apparatus fitted with a reflux condenser by setting the temperature at 240 °C and digested for 2:30 hours. The

digest was allowed to cool to room temperature for 10 minutes without dismantling the condenser and for 10 minutes after removing the condenser.

To the cooled solution, 10 mL of distilled water was added to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digested residue while filtering with filter paper (Whatman 125 mm diameter, Germany) into 100 mL volumetric flask. The round bottom flask was rinsed subsequently with distilled water and added into the filter paper. Then finally, the solution in the volumetric flask was filled to the mark (100 mL) using distilled water. The digestions of samples were carried out in triplicate for each sample. Similarly, digestions of the blanks were performed in parallel with the milk samples keeping all digestion parameters the same. The metal concentrations in the digested sample solutions were determined by using AAS.

3.3 Apparatus and Instrument

The apparatus that was used during the study included: Crucible, pH-013M Portable pH Meter, Oven, volumetric flask, desiccator, beakers, muffle furnace, pair of thongs, conical flask, burette, measuring cylinders, funnel, filter papers, pipettes, and micropipettes (Pyrex, USA) to dilute sample solutions and prepare standard solutions, round bottom flasks 250 mL were used to digest pasteurized cow milk samples, spiked cow milk samples, and blank solutions. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the collected samples and digested samples until analysis. A flame atomic Absorption Spectrophotometer (BUCK SCIENTIFIC MODEL 210VGP) equipped with deuterium arc background correctors provided with air-acetylene flame was used for the analysis of the digested pasteurized cow milk samples for the selected minerals.

3.4 Chemicals and Standards

Nitric acid (70%) and Perchloric acid (70%) were used for the digestion of the pasteurized cow milk samples; 0.1 N NaOH solution to determine titratability acidity; Stock standard solutions (1,000 ppm) of each element used to prepare standard solutions and hence construct calibration curve; Distilled water was used for all dilutions.

3.5 Determination of Selected Physicochemical Parameters

3.5.1 Determination of pH

The pH of pasteurized milk samples were determined using the Portable pH Meter (PH-013M) after the pH meter was calibrated with standard buffers solution pH= 4.0 and pH=7.0. Then, 50

mL of pasteurized cow milk sample was placed in a beaker and the sensor electrode of the pH meter (pH electrode) was immersed into the beaker and pH was read out.

The sensor of the electrode of the pH meter was cleaned with distilled water between measuring the different pasteurized cow milk products samples.

3.5.2 Titratable Acidity of Milk

Titratable acidity of the milk samples was expressed as percent lactic acid [131]. a 10 mL pasteurized milk sample was pipetted out in a 100 mL conical flask and 3 to 5 drops of 1% phenolphthalein indicator solution was added into it and diluted with 10 mL of distilled water. The mixture was then titrated with 0.1 N NaOH solutions until a faint pink color appeared. The volume of NaOH consumed during the titration was recorded and used to calculate the Titratable acidity (%) as follows:

$$\text{Titratable acidity \% (as lactic acid)} = \frac{(\text{Vol.of 0.1 N NaOH (mL) x0.009})}{\text{weight of milk sample}} \times 100 \qquad 3.1$$

3.5.3 Determination of Moisture Content

The determination of moisture in pasteurized milk samples was done according to the procedure mentioned in IS 16072 (2012) standard [130]. Three crucibles and its lid were placed in the oven at 102 ± 2 ℃ for 1 hr. Then, the crucibles were covered with their lids and transferred from the oven to the desiccator to cool to room temperature and then labeled and weighed (W1). Approximately 10 mL of pasteurized cow milk samples were poured in each crucible, and covered with their lid and weighed the covered crucibles accurately and quickly (W2). Then, the crucible was uncovered and put them and their lids in the oven at 102 ± 2 ℃ for 3 hrs. They were removed and transferred to desiccators to cool, finally weighed (W3). For each sample, three replicate determinations were made and the mean value was recorded. The moisture content on a wet-weight basis was calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{W2-W3}{W2-W1} \times 100 \qquad 3.2$$

Where, W1 = weight of crucible with lid; W2 = weight of crucible with lid and sample before drying; and W3 = weight of crucible with lid and sample after drying.

3.5.4 Determination of Total Solids

Determination of total solids content in pasteurized cow milk samples was done using the Gravimetric Method [134]. To determine the total solids, 5 mL thoroughly mixed pasteurized milk sample was transferred to a pre-weighed flat bottom dish.

Then, the milk sample was then dried in a forced-air oven at 102 ± 2 ℃ for 3 hrs. Then, the dried samples were taken out of the oven and placed in desiccators and the dry sample was weighed. The heating, cooling, and weighing processes were repeated until constant weight was achieved. The calculation of total solids is the difference in mass of dish with dried sample (M2) and mass of dish (M3) divided by the difference in mass of dish with sample (M1) and mass of dish (M3) and expressed in percentage.

$$\text{Total solids (\%)} = \frac{W2-W3}{W1-W3} \times 100 = \frac{\text{weight of dried sample (g)}}{\text{weight of milk sample (g)}} \times 100 \qquad 3.3$$

Where, W1 = mass in g of dish and sample; W2 = mass in g of the dish and dried sample; and W3 = mass in g of dish.

Total solids can also be calculated as 100% minus the percent moisture content.

3.5.5 Determination of Total ash Content

Determination of ash content (mineral contents) in pasteurized cow milk was done using the Gravimetric Method [137]. The dried milk samples used for determination of total solids content were ignited in a muffle furnace at a temperature of 550 ℃ for 3 hours for ashing. Other volatile materials were vaporized and organic substances were burnt to ashes. The ash was cooled in desiccators, and then their weight was determined. The percentage ash content was calculated as follows:

$$\text{Total ash (\%)} = \frac{\text{Weight of residue}}{\text{weight of sample}} \times 100 \qquad 3.4$$

3.6. Determination of Mineral Content of Milk Sample

The mineral contents of the pasteurized cow milk samples were determined by atomic absorption spectrometry according to the methods of AOAC [154]. The digested milk samples were analyzed to determine the concentration of Ca, Mg, Cu, Mn, Ni, Co, Cr, and Pb using Flame

Atomic Absorption Spectrophotometry ((BUCK SCIENTIFIC MODEL 210VGP after a standard curve was prepared for each elements using standard stock solution the element to be analyzed.

3.6.1. Determination of Ca, Mg, Cu, Mn, Ni, Co, Cr, and Pb by FAAS

In this technique the atoms of an element are vaporized and atomized in the flame. The atoms then absorb the light at a characteristic wavelength. The source of the light is a hollow cathode lamp, which is made up of the same element, which has to be determined.

The lamp produces radiation of an appropriate wavelength, which while passing through the flame is absorbed by the free atoms of the sample. The absorbed energy is measured by a photo-detector read-out system. The amount of energy absorbed is proportional to the concentration of the element in the sample. The digested pasteurized milk samples were analyzed for its elemental contents by Flame Atomic Absorption Spectrophotometer–Buck Scientific (Model 210 VGP). In this technique, different Hollow cathode lamps were used for each element and then the instrument was run first by aspirating working standard solutions of each metal before the determination of each metal in the digested milk samples. A series of working standard solutions were prepared from 10mg/L intermediate standard solutions of their respective metals, which were prepared from the stock standard solutions containing1000mg/L. For the determination of Ca and Mg, further dilution of the original solution was done by using 5.0 mL original solution and enough distilled water was added to it to make the volume up to 100 mL. The concentrations of elements recorded were in terms of ppm.

3.7 Instrument Operating conditions and Calibration

3.7.1 Instrument Operating Condition of FAAS

For the determination of elements such as Ca, Mg, Cu, Mn, Ni, Co, Cr, and Pb was determined after instrument parameters such as lamp alignment, slit width, and wavelength were adjusted according to the requirement of the instrument after the respective hollow cathode lamp was inserted into the turret of the atomic absorption spectrophotometer. Table 3.2 showed the instrumental conditions for each element analyzed.

Table 3.2: FAAS instrumental operating conditions for the determination of selected macro and micronutrient elements and heavy metals

Elements	**Ca**	**Mg**	**Cu**	**Mn**	**Ni**	**Co**	**Cr**	**Pb**
Wavelength (nm)	422.7	285.2	324.7	279.5	239.7	240.7	357.9	283.2
Slit width (nm)	0.7	0.7	0.7	0.7	0.2	0.2	0.7	0.7
Lamp current (mA)	2.0	1.0	1.5	3.0	3.0	4.5	3.0	2.0

3.7.2 Calibration of FAAS

Atomic absorption spectrophotometer calibration for each metal analysis was carried out by preparing four concentrations of working standard solutions from Stock standard solutions (1,000 ppm) of each element. The four concentrations of working standard solutions used for each metal are shown in Table 3.3.

Table 3.3: Concentrations of working standards for each metal analyzed using FAAS

Metals	**Concentration of the standards (mg/L)**
Ca	0.15; 1.0; 2.0; 4.0
Mg	1.0; 2.0; 4.0; 6.0
Cu	0.01; 0.5; 1.5; 2.0
Mn	0.05; 0.1; 0.3; 0.5
Co	0.06; 0.5; 1.0; 2.0
Cr	0.05; 0.5; 1.0; 2.0
Ni	0.06; 0.1; 0.5; 1.0

Pb	0.09; 0.1, 0.5; 1.0

3.8 Method validation

3.8.1 The method detection limit (MDL) for FAAS

The method detection limits for each metal were estimated using blank digest for selected pasteurized milk sample. Each blank solution was run with FAAS for the level of the metal in a similar manner as the samples and triplicate readings were recorded. Then, the standard deviations of the blank concentrations were calculated and used to calculate the method detection limit as follows, [159]

MDL =3×Standard deviation of the blank

3.8.2 Recovery Test

Since certified standard reference materials were not available in the laboratory, the validity of the analytical procedures and efficiency of FAAS used for sample analysis in this work was verified by spiking experiments and calculating the recovery (%) [160].

A spiking experiment was done using one of the milk samples, to evaluate or check the validity of the digestion procedure and efficiency of the atomic absorption spectrophotometer used for sample analysis. All the spiked samples were digested in triplicate following the same digestion procedure used for unspiked pasteurized milk samples. The digested spiked milk samples were analyzed for their respective metal content using FAAS. In a digestion flask containing a 5.0 mL of pasteurized milk sample, 0.5 mL of a 1000 mg/L stock solution of Ca, Mg, Cu, Mn, Ni, Co, Cr, and Pb were spiked at once and digested in a similar procedure used for the digestion of milk samples and it was carried out in triplicate. Then, the digested spiked samples were analyzed for the respective metals by FAAS. The %recovery for each metal was calculated using the following formula:

%Recovery= (Amount recovered)/ (Spiked amount) ×100

3.9 Statistical Analysis or Data Analysis

The data was analyzed using SPSS version 16.0, Origion8, and Microsoft excel program. The Mean, Standard deviation, %RSD, and one-way analysis of variance (ANOVA) were done using the above statistical analysis tools to examine the statistical significance of differences in the mean concentration of the proximate and mineral compositions of the different pasteurized cow milk samples studied. The mean values of nine replicates per milk sample type were compared using the least significant difference (LSD) test at $P < 0.05$ probability levels.

4 RESULTS AND DISCUSSIONS

4.1 Results of the physicochemical parameters of pasteurized milk samples

The results of the physicochemical parameter of pasteurized cow's milk samples were presented in Tables 4.1.

Table 4.1: Physicochemical composition of pasteurized milk samples (mean ± SD, n= 3, %) except for pH

Milk sample codes	**Physicochemical parameters**				
	pH	Moisture (%)	Total solid (%)	Total ash (%)	Titratable acidity (%)
AA/B 1	6.24 ± 0.01	86.86 ± 1.19	13.14 ± 1.19	0.715 ± 0.009	0.356 ± 0.003
AA/B 2	6.05 ± 0.02	90.61 ± 0.56	9.39 ± 0.56	0.661 ± 0.030	0.310 ± 0.001
AA/B 3	5.87 ± 0.06	91.50 ± 0.47	8.50 ± 0.47	0.606 ± 0.067	0.234 ± 0.006
AA/B 4	6.24 ± 0.02	88.44 ± 0.37	11.56 ± 0.37	0.723 ± 0.002	0.231 ± 0.005
AA/B 5	6.41 ± 0.01	85.10 ± 0.42	14.90 ± 0.42	0.703 ± 0.048	0.263 ± 0.008
AA/B 6	6.32 ± 0.01	87.41 ± 0.36	12.59 ± 0.36	0.714 ± 0.002	0.408 ± 0.005
AA/B 7	5.87 ± 0.06	91.67 ± 0.32	8.33 ± 0.32	0.646 ± 0.007	0.243 ± 0.002

pH

pH is a physicochemical property that indicates the bitterness or sourness of milk and their products. In the present study, the average pH value of the pasteurized milk samples ranged from 5.87 to 6.41. The highest mean value (6.41) of pH was recorded in AA/B5 milk sample, while the least mean value (5.87) of pH was recorded in AA/B3 and AA/B7 samples (Table 4.1). One-Way ANOVA analysis showed that the pH content was significantly different, F (6, 14) =

132.668, $P < 0.001$, among the pasteurized milk samples. However, Post hoc test revealed the pH value of samples AA/B1, AA/B4, and AA/B6 were not significantly different. Similarly, the pH value of samples AA/B3 and AA/B7 were not significantly different. The results of ANOVA analysis for each physicochemical parameter were shown in appendix II.

The result showed that the mean pH values of all pasteurized milk samples were below the lower limit of pH value that ranges from pH 6.6 to 6.8 for fresh cow milk [161, 162]. Lower pH value indicates the pH of milk changes over time, as milk goes sour, it becomes more acidic and the pH gets lower. This occurs as bacteria in milk convert lactose into lactic acid. Furthermore, a pH values greater than 6.8 indicate that the milk has coagulated or mastitis and a value below 6.6 indicate the presence of colostrum or bacterial contamination [162]. The pH value in this study for all pasteurized milk samples were below the pH value reported by Abou- Arab, A. A. K., *et al.*, who reported a pH range of 6.60-6.70 [163].

Moisture content (%)

The average moisture content of pasteurized milk samples analyzed was varied from 85.10% to 91.67%. The highest value was in sample AA/B7 and the lowest was in sample AA/B5 (Table 4.1). One-Way ANOVA analysis showed that there was a significant difference among the seven pasteurized milk samples on the values of moisture content (%), $F(6, 14) = 49.180$, $P < 0.001$. Post hoc test revealed the moisture content (%) of samples AA/B1, AA/B4, and AA/B6 were not significantly different. Similarly, the moisture content (%) of samples AA/B2, AA/B3, and AA/B7 were not significantly different. Abou- Arab, A. A. K., *et al.*, [162] reported that the moisture content (%) content of pasteurized milk samples collected from local markets, Cairo Governorate, Egypt were ranged from 88.15% to 88.75%. Similar study by Hossain, T.J. *et al.*, [164] reported moisture content (%) content of pasteurized milk samples collected from different retail markets in Bangladesh were ranged from 89.0% to 90.83%, while Saha, S. and A. Ara, A. [165] were reported a moisture content of different brands of pasteurized milk samples available in Sylhet City of Bangladesh ranged from 88.42% to 88.65%. Generally, milk is approximately 87% water [29], so it is a good source of water in the diet. However, an increase in moisture content (%) of milk might be due to the mixing of water with the raw milk. This malpractice decreases the nutritional value of milk [166] and it may cause serious health hazards if added

water is contaminated with pathogens, metals, etc., for the consumers [167]. Therefore, samples with water content of more than 90% might be regarded as adulterated with water.

Total solid (%)

The average total solid content of pasteurized milk samples analyzed was varied from 8.33% to 14.90% (Table 4.1). The highest value was in sample AA/B5 and the lowest was in sample AA/B7. One-Way ANOVA analysis showed that there was a significant difference among the seven pasteurized milk samples on the values of total solid content (%), $F(6, 14) = 49.180$, $P < 0.001$. However, Post hoc test revealed the total solid content (%) of samples AA/B2, AA/B3, and AA/B7 were not significantly different. Similarly, the total solid content (%) of samples AA/B1, AA/B4, and AA/B6 were not significantly different. According to European Union established standards for the total solids content of cow milk is not to be less than 12.5% FAOSTAT, [168]. Therefore, in this study four pasteurized samples were found to have an average total solid content that were below the recommended standards. The lower total solid content of the milk samples might be due to the addition of water with milk, and lower fat content and this might also be due to difference in breed, feeding and management practices which have important effects on milk composition and quality [162].

Total ash content (%)

The average amount of total ash in pasteurized milk samples analyzed were in the ranged of 0.606% to 0.723% (Table 4.1). The highest value was in sample AA/B4 and the lowest was in sample AA/B3. One-Way ANOVA analysis showed that there was no significant difference among the seven pasteurized milk samples on the values of total ash content (%), $F(6, 14) = 5.235$, $P = 0.005$. However, Post hoc test revealed the total ash content (%) of samples AA/B2, AA/B3, and AA/B7 were not significantly different. Similarly, the total solid content (%) of all milk samples was not significantly different except for sample AA/B3.

The ash content of the pasteurized milk samples varied from 0.606% to 0.723%, which falls within the usual range of the ash content of the raw milk 0.6 to 0.9% [149]. Furthermore, the amount of ash in the pasteurized milk samples AA/B2, AA/B3 and AA/B7 were in conformity as found by Hossain, *et al.*, [164] as 0.64%–0.71%, while the amount of ash in the pasteurized milk

samples AA/B1, AA/B4, AA/B5, and AA/B6 were in conformity as found by Abou- Arab, A. A. K., *et al.*, and Karmaker, A, *et al.*, [162, 169] as 0.70%–0.95% and 0.70%-0.74% respectively which satisfied the standard (≥ 0.70%) provided by Bangladesh Standards and Testing Institution (BSTI) [170] for pasteurized milk. However, pasteurized milk samples such as AA/B3, AA/B2 and AA/B7 contained ash content that is less than the BSTI (2002) standard [170], which demands at least 0.7% of ash for the pasteurized milk.

Titratable acidity (%)

Titratable acidity measures the freshness, bacterial activity, and taste of milk and acknowledged as an indicator of milk quality. Popescu and Angel stated that the high quality milk should have maximum acidity of 0.14% [171]. In this study, it was found that the pasteurized milk samples had the titratable acidity ranges from 0.231%–0.408% (Table 4.1). The highest value was in sample AA/B6 and the lowest was in sample AA/B4. One-Way ANOVA analysis showed that there was significant difference among the seven pasteurized milk samples on the values of titratable acidity (%), $F(6, 14) = 600.642$, $P < 0.001$. However, Post hoc test revealed that the titratable acidity of samples AA/B3, AA/B4, and AA/B7 were not significantly different.

The titratable acidity values of all the pasteurized milk samples analyzed were very much higher than the acidity limit specified BSTI standard for pasteurized milk [170] and were reasonably higher than reported acidity values for pasteurized milk samples [163, 164, 165, 169], suggesting deterioration in the quality of the pasteurized milk samples. These might be due to the uncleanliness during milking collection and production in the Dairy factories or it might be due to temperature effect during sample transportation from Addis Ababa to Hawassa University.

Table 4.2: Comparison of concentration of metals in cow milk of present study with different countries and FAO/WHO

Country	**Physicochemical properties**					
	pH	Moisture (%)	Total solid (%)	Total ash (%)	Titratable acidity (%)	Referance
Egypt	6.6 -6.7	88.15 -88.75	11.25-11.85	0.70-0.95	0.15-0.17	163
Bangladesh	-	89.0-90.83	9.17-11.0	0.64–0.71	0.144-0.162	164
Bangladesh	-	88.42-88.65	11.35-11.58	-	0.136-0.169	165
FAO/WHO	6.6- 6.8	87-88	11	0.7 – 0.8	< 0.15	
Ethiopia	5.81-6.42	84.78-91.91	8.09-15.16	0.546-0.739	0.228-0.411	Present Study

4.2 Results of optimization of the acid digestion procedure

The experimental variables that were attempted during optimization of the digestion of different amounts of pasteurized milk samples were shown in appendix III. Optimized procedures were selected based on the usage of lesser sample and reagent volume, shorter digestion time and reasonable mild temperature for obtaining clear and colorless solutions of the resulting digests. Based on this fact the optimized digestion conditions for the pasteurized cow milk samples in

this study were (5.0 mL HNO_3 (70%): 3.0 mL $HClO_4$ (70%) volume ratio of reagents, 240 °C digestion temperature and 2:30 hours digestion time for the digestion of 5.0 mL of milk samples.

4.3 Calibration curve for each element analyzed

The correlation coefficients for the calibration curves for all the selected metals analyzed using FAAS were greater than or equal to 0.999 which assured the linearity of instrumental response for individual metals or these correlation coefficients showed that there was a very good correlation (linear relationship) between concentration and absorbance.

The equation of the graph and its R^2 value for each metal are shown in Table 4.2. The calibration graphs for the selected metal analyzed were shown in appendix IV.

Table 4.3: Calibration graph equation and R^2 value

Metal	**Calibration Equation**	**R^2 Value**
Ca	Y= 0.0506x - 0.0026	0.9999
Mg	Y = 0.0016x + 0.0001	0.9995
Cu	Y = 0.0518x + 0.0012	0.9996
Mn	Y = 0.1139x - 0.0011	0.9994
Ni	Y = 0.027x + 0.0005	0.9993
Co	Y = 0.0435x - 0.00031	0.999
Cr	Y = 0.0751x + 0.0005	0.9993
Pb	Y = 0.0276x - 0.0003	0.9998

4.4 Method Detection Limits Values

The method detection limit values for each element analyzed in pasteurized milk samples were shown in Table 4.4 and they were above the detection limit of the instrument.

Table 4.4: Method detection limit for the elements determined in milk samples

Element	Ca	Mg	Cu	Mn	Ni	Co	Cr	Pb
IDL mg/L	0.05	0.005	0.005	0.03	0.05	0.05	0.04	0.08
MDL mg/L	0.5	0.05	0.01	0.05	0.06	0.06	0.05	0.09

The method detection limits estimated indicated that if the concentration of the respective element is at least equal to the detection limit, it can be detected but not quantified.

4.5 Results of the mineral contents of pasteurized milk samples

The results of the concentration of the elements analyzed for each type of pasteurized milk samples were presented in Tables 4.5.

Table 4.5: Results of the concentration of the elements (mean + SD, n = 9, mg/L) determined in pasteurized cow milk samples

Sample code	Ca	Mg	Mn	Ni
AA/B 1	1130.33 ± 1.80	118.33 ± 1.50	0.071 ± 0. 001	ND
AA/B 2	1471.78 ± 1.72	98.11 ± 1.83	0.099 ± 0.001	0.068 ± 0.001
AA/B 3	1041.5 ± 0.7	73.56 ± 1.59	0.092 ± 0.001	0.068 ± 0.001
AA/B 4	1114.78 ± 2.77	113.56 ± 1.94	0.052 ± 0.001	ND
AA/B 5	1129.78 ± 3.23	102.33 ± 2.06	0.102 ± 0.001	0.068 ± 0.001
AA/B 6	1495.11 ± 1.76	119.00 ± 1.73	0.072 ± 0.001	ND

AA/B 7	1603.89 ± 2.9	82.78 ± 1.86	0. 085 ± 0.001	0. 060 ± 0.001

Sample code	**Cu**	**Co**	**Cr**	**Pb**
AA/B 1	0.060 ± 0.003	0.071 ± 0.001	0.072 ± 0.001	ND
AA/B 2	0.035 ± 0.001	0.063 ± 0.001	0.056 ± 0.001	ND
AA/B 3	0.069 ± 0.001	0.079 ± 0.001	0.065 ± 0.001	ND
AA/B 4	0.082 ± 0.001	0.058 ± 0.001	0.053 ± 0.001	ND
AA/B 5	0.076 ± 0.001	0.081 ± 0.001	0.081 ± 0.001	ND
AA/B 6	0.054 ± 0.001	0.071 ± 0.001	0.075 ± 0.001	ND
AA/B 7	0.079 ± 0.001	0.067 ± 0.001	0.071 ± 0.001	ND

Note that ND =not detected

Ca, Mg, Mn, Cu, Co, and Cr were present in all pasteurized milk samples while the concentration of Ni was also found to be below the detection limits for pasteurized milk samples such as AA/B1, AA/B4, and AA/B6. However, the concentration of Pb was found to be below the detection limits for all pasteurized milk samples.

Calcium

The mean calcium concentration in the analyzed pasteurized milk samples ranged from 1603.89 to 1041.5 mg/L (Table 4.5). The highest mean calcium content were found in AA/B7 milk sample and the lowest mean calcium content (1041.5 mg/L) was found in AA/B 3 milk sample. One-Way ANOVA analysis showed that there was a significant difference in the calcium content, $F(6, 56) = 82308.459$, $P < 0.001$, among the pasteurized milk samples. However, Post hoc testing revealed that the mean calcium concentration obtained for milk samples AA/B1 and milk samples AA/B5 were statistically similar. The results of ANOVA analysis for the selected

metals were shown in appendix V. The mean calcium concentration of pasteurized milk samples AA/B2, AA/B 6, and AA/B7 were above the reported average calcium concentration (1200 mg/L) [172, 173].

Magnesium

The mean magnesium concentration in the analysed pasteurized milk samples ranged from 73.56 to 119.00 mg/L (Table 4.5). The highest mean magnesium content were found in AA/B 6 milk sample and the lowest mean magnesium content was found in AA/B3 milk sample. One-Way ANOVA analysis showed that there was a significant difference in the magnesium content, $F(6, 56) = 871.812$, $P < 0.001$, among the pasteurized milk samples. However, Post hoc testing revealed that the mean magnesium concentration obtained for milk samples AA/B1 and milk samples AA/B6 were statistically similar. The mean magnesium concentration of all pasteurized milk samples were below the reported average magnesium concentration (128 mg/L) [172, 173].

Copper

The mean copper concentration in the analyzed pasteurized milk samples ranged from 0.035 to 0.082 mg/L (Table 4.4). The highest mean copper content were found in AA/B4 milk sample and the lowest mean copper content was found in AA/B2 milk sample. One-Way ANOVA analysis showed that there was a significant difference in the copper content, $F(6, 56) = 1562.193$, $P < 0.001$, among the pasteurized milk samples.

The results of the copper content of the pasteurized milk samples were far below the results reported *by* Abou- Arab *et al.*, [163] as 0.12–0.16 mg/L. The results obtained for copper found to exceed the maximum limit of Cu in milk samples recommended by Standardization Administration of the People's Republic of China (0.01 mg/L) [174] and WHO/FAO (0.05 mg/L) [175], which might be attributed to environmental contamination [1, 176, 177].

Manganese

The mean manganese concentration in the analyzed pasteurized milk samples ranged from 0.052 to 0.102 mg/L (Table 4.5). The highest mean manganese content were found in AA/B5 milk sample and the lowest mean manganese content was found in AA/B4 milk sample. One-Way ANOVA analysis showed that there was a significant difference in the manganese content, $F(6, 56) = 14394.085$, $P < 0.001$, among the pasteurized milk samples. However, Post hoc testing revealed that the mean manganese concentration obtained for milk samples AA/B1 and milk samples AA/B6 were statistically similar. The results of the manganese content of the pasteurized milk samples were higher than the results reported by Abou- Arab *et al.*, [163] as 0.03–0.07 mg/L, which was higher than typical ranges of manganese concentrations in milk and milk products, 0.02–0.49 mg/L [178]. These values of manganese exceed the maximum limit for manganese guideline values based on the World Health Organization [179]. The high concentration of manganese might be due to diet enrichment of lactating cows with Mn-containing salts to compensate for mineral deficiencies, the use of feeds high in organic Mn (beets, squash and tulip bulbs) and the use of deep wells.

Nickel

The mean nickel concentration of pasteurized milk samples such as AA/B2, AA/B3, and AA/B5 were the same and it was found to be 0.068 mg/L. However, the mean nickel concentrations for AA/B7 pasteurized milk samples were found to be 0.060 mg/L. One-Way ANOVA analysis showed that there was a significant difference in the nickel content, $F(6, 56) = 2952.799$, $P < 0.001$, among the pasteurized milk samples. The results of the nickel content of the pasteurized milk samples varied from 0.060 to 0.068 mg/L, which is in a good agreement with the range of the nickel content of the pasteurized milk reported by Abou-Arab, *et al.*, 0.02 to 0.06 mg/L [163]. These results of nickel were below the maximum acceptable limit for nickel [180].

Cobalt

The mean cobalt concentration in the analysed pasteurized milk samples ranged from 0.058 to 0.081 mg/L (Table 4.4). The highest mean cobalt content were found in AA/B5 milk sample and the lowest mean cobalt content was found in AA/B4 milk sample. One-Way ANOVA analysis

showed that there was a significant difference in the cobalt content, $F(6, 56) = 194.227$, $P < 0.001$, among the pasteurized milk samples. However, Post hoc testing revealed that the mean cobalt concentration obtained for milk samples AA/B1 and AA/B6 and milk samples AA/B3 and AA/B5 were statistically similar. The results of the cobalt content of the pasteurized milk samples were higher than the results reported by Abou- Arab, *et al.*, [163] as 0.020–0.04 mg/L. Furthermore, the mean cobalt concentrations were found to exceed the maximum limit for cobalt guideline values based on the World Health Organization [179]. The high amount of cobalt might be due to environmental pollution such as the use of polluted water to irrigate grass used for grazing cattle and production of hay's for use as fodder.

Chromium

The mean chromium concentration in the analyzed pasteurized milk samples ranged from 0.053 to 0.081 mg/L (Table 4.5). The highest mean chromium content were found in AA/B5 milk sample and the lowest mean chromium content was found in AA/B4 milk sample. One-Way ANOVA analysis showed that there was a significant difference in the chromium, $F(6, 56) = 15356.008$, $P < 0.001$, among the pasteurized milk samples. The results of the chromium content of the pasteurized milk samples were higher than the results reported by Abou- Arab, *et al.*, [163] as 0.01–0.07 mg/L, which exceeded the maximum permissible limit of chromium guideline values based on the World Health Organization [179]. This might be due to environmental (diet, season) factors as well as using agricultural chemicals, diet enrichment of lactating cows with Cr-containing salts to compensate for mineral deficiencies, and Cr contamination during milk processing [180].

4.6 Results of the Recovery experiment

Recovery experiment was carried out using selected pasteurized cow milk sample (AA/B3). The results of the %recovery experiment for the analyzed elements were shown in Table 4.6.

Table 4.6: Recovery test results (mean ± SD, n=9, mg/L) for the analyzed elements in pasteurized milk sample AA/B3.

Metal	Conc. of unspiked sample (mg/L)	Amount added (0.5 mg/L)	Conc. Spiked sample (mg/L)	Recovery (%)
Ca	1041.5 ± 0.7	0.5	1042.02 ± 0.03	104.0
Mg	73.56 ± 1.59	0.5	74.05 ± 4.31	98.0
Cu	0.069 ± 0.001	0.5	0.6396 ± 0.0564	114.1
Mn	0.092 ± 0.001	0.5	0.64 ± 0.072	109.6
Ni	0.068 ± 0.001	0.5	0.573± 0.007	101.0
Co	0.079 ± 0.001	0.5	0.5696 ±0.0145	98.1
Cr	0.065 ± 0.001	0.5	0.5765 ±0.0158	102.3

The %recovery values for all the analysed elements lie within the range 98.0% to 114.1%, which is in the acceptable range (80–120 %), which suggested that the experimental procedures and the method of analysis were accurate and valid.

5 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Physicochemical quality parameters such pH, moisture, total solids, total ash, titratable acidity, and minerals such as (Ca, and Mg), trace elements (Cu, Mn, Ni, and Co) and heavy metals (Cr and Pb) were analyzed to evaluate the quality of seven pasteurized cow's milk samples bought from the local markets in Addis Ababa, Ethiopia. The result showed that the mean pH values of all pasteurized milk samples were below the lower limit of pH ranges for fresh cow milk, which is from pH 6.6 to 6.8 suggesting the pasteurized milk samples goes sour, and become more acidic during transportation. Some samples contained over 90% water which might indicate the mixing of water with the milk sample. Similarly, four pasteurized samples were found to contain an average total solid content that were below the recommended standards (12.5%). These might be due to the addition of water with milk, and the lower fat content which arises from the difference in breed, feeding and management practices. Four pasteurized milk samples were found to satisfy the demand that pasteurized milk should at least contained 0.7% ash. The titratable acidity values of all the pasteurized milk samples analyzed were very much higher than the acidity limit specified BSTI standard for pasteurized milk, suggesting the deterioration in the quality of the pasteurized milk samples. essential minerals like calcium (1041.5 -1603.89 mg/L) and magnesium (73.56 - 119.00 mg/L) were within acceptable ranges, but trace elements copper, manganese, cobalt and chromium exceeded WHO/FAO safety limits although nickel levels were safe lead was not detect further investigation into the elevated trace metals is recommended, though these findings do not necessarily deem the milk unfit for consumption.

5.2 RECOMMENDATION

Special attention should be given to the level of metals in cow's milk. If they are accumulated in concentrations greater than their permissible limit, they can lead to adverse health effects. Therefore, further studies on common cow's food stuffs and underground water from the study areas are required to identify the case of some elevated metal levels in cow's milk. Efficiency of other extractants like HNO_3:H_2SO_4, HNO_3: $HClO_4$, H_2SO_4:$HClO_4$ should also be checked in the determinations of the essential and toxic metals in the cow's milk using different digestion methods like microwave and dry-ashing method. The levels of metals were determined using FAAS. In addition to FAAS other highly sensitive instruments like atomic emission spectrometer (AES), inductively coupled plasma mass spectrometer (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP OES) could be used to check the levels of these metals.

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APPENDICES

Appendex I: Major private dairy enterprises operating in different parts of Ethiopia

Ser. №	Dairy Enterprise	Location	Year of establishment	Daily processing capacity (Liters)	Attained average capacity (Liters)
1	Sebeta Agro Industry (Mama Dairy)	Sebeta	1998	35000	30000
2	Lame Dairy Processing	Addis Ababa	2008	60000	30000
3	Diredawa Dairy Processing Enterprise	Dire Dawa	1972	20000	20000
4	MB PLC (Family Milk)	Addis Ababa	2003	15000	7000
5	Yadeni Dairy Farm (Bora Milk)	Addis Ababa	2008	15000	7000
6	Ada'a Dairy Cooperative	Debre Zeit	1998	15000	3000
7	Lemma Dairy	Debre Zeit	2004	10000	3000
8	Berta and Family plc	Addis Ababa	2000	9000	6000
9	Genesis Farm	Debre Zeit	2001	4000	4000
10	Holland Dairy	Debre Zeit		4000	4000
11	Almi Tiku Wetet (Almi Fresh Milk)	Hawassa		4000	3000
12	Ruth and Hirut Dairy Farm	Addis Ababa	2008	4000	1500
13	Abay fana Awash Agro Industry	Adama		3500	2000

14	Chuye milk and milk products processing	Addis Ababa		3000	1000
15	Fantu and Family Dairy Farm	Addis Ababa		2500	2000
16	Zemen Milk	Mekelle		2000	150
17	Pinguine International Businesses plc (Cheese world)	Addis Ababa		1800	600
18	Life Milk Processing Enterprise	Sululta		1500	1500
19	Semit Agro-Industry (Enat Milk)	Mojjo			6000
20	Beral Milk	Addis Ababa	1991		4000
21	Harmonius Agro-Industry	Adama			4000
22	Jantekel Dairy Union (Facil Milk)	Gondar		1200	300

Source: Current study survey result; Land O' Lakes, 2011)

Appendix II: ANOVA analysis results for selected physicochemical parameters

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total solids in %	Between Groups	115.998	6	19.333	49.180	.000
	Within Groups	5.504	14	.393		
	Total	121.501	20			
Total ash content in %	Between Groups	.035	6	.006	5.235	.005
	Within Groups	.016	14	.001		
	Total	.051	20			
Titratable acidity as % lactic acid	Between Groups	.085	6	.014	600.642	.000
	Within Groups	.000	14	.000		
	Total	.085	20			
Moisture content in %	Between Groups	115.998	6	19.333	49.180	.000
	Within Groups	5.504	14	.393		
	Total	121.501	20			
pH	Between Groups	.830	6	.138	132.668	.000
	Within Groups	.015	14	.001		
	Total	.845	20			

Appendix III: reagents ratios, temperature and time attempted during optimization of digestion of milk samples. Optimization for reagent volume

Trials	**Reagent volume (mL)**		**Sample volume (mL)**	**Temperature (°C)**	**Time (hour)**	**Observations**
	HNO_3	$HClO_4$				
1	3	1	5	200	2:00	Deep yellow
2	3	3	5	200	2:00	Yellow
3	4	2	5	200	2:00	Light yellow
4	4	2	5	200	2:00	Clear and yellow
5	5	2	5	200	2:00	Almost clear
6	5*	3*	5	200	2:00	Clear & colorless*

Optimization for temperature

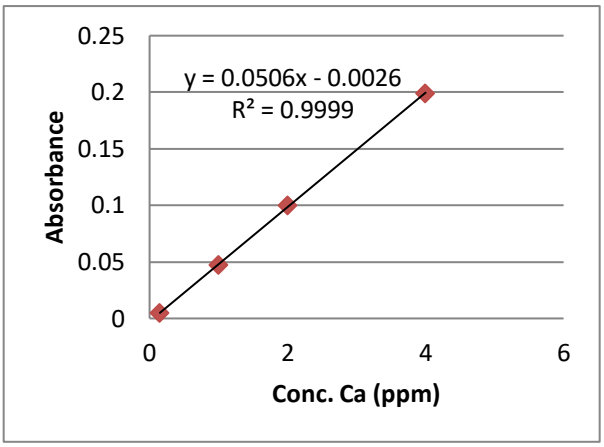
Trials	**Reagent volume (mL)**		**Sample volume (mL)**	**Temperature (°C)**	**Time (hour)**	**Observations**
	HNO3	HClO4				
1	5	3	5	120	2:00	Deep yellow
2	5	3	5	140	2:00	Yellow
3	5	3	5	160	2:00	Light yellow
4	5	3	5	180	2:00	Clear and yellow
5	5	3	5	200*	2:00	Clear & colorless*
6	5	3	5	220	2:00	Clear & colorless

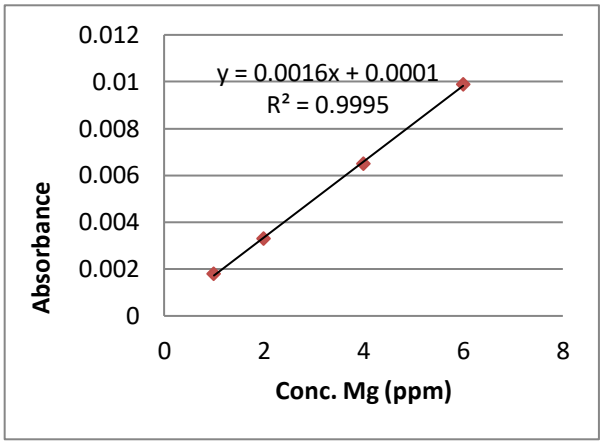
Optimization for time

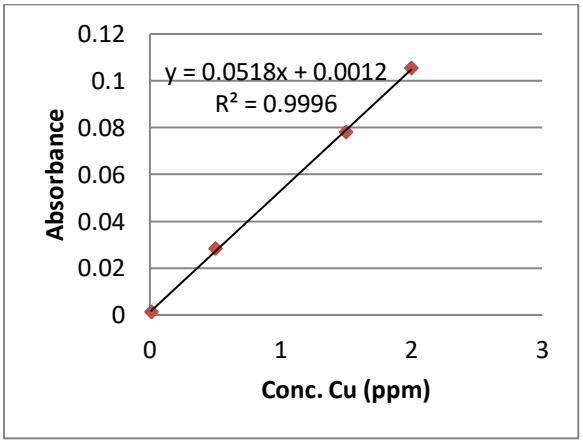
Trials	**Reagent volume (mL)**		**Sample volume (mL)**	**Temperature (°C)**	**Time (hour)**	**Observations**
	HNO3	HClO4				
1	5	3	5	120	1:00	Deep yellow
2	5	3	5	140	1:30	Yellow
3	5	3	5	160	1:45	Clear and yellow
4	5	3	5	180	2:00*	Clear & colorless*
5	5	3	5	200	2:15	Clear & colorless
6	5	3	5	220	2:30	Clear & colorless

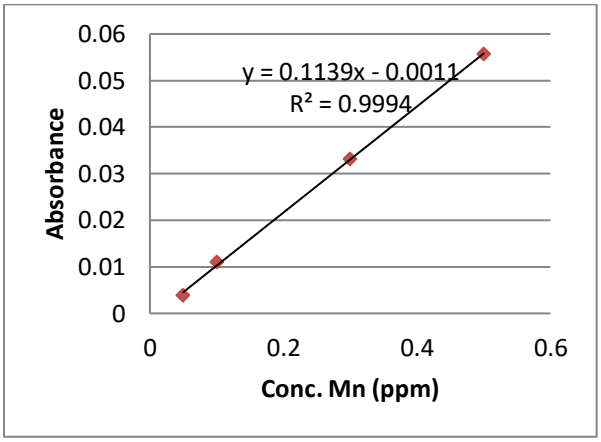
*The optimized conditions for the three parameters (reagents volume ratio, time and temperature).

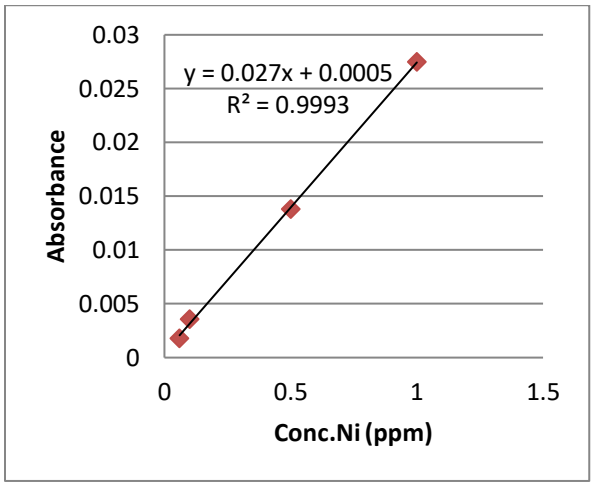
Appendix IV: Calibration graphs

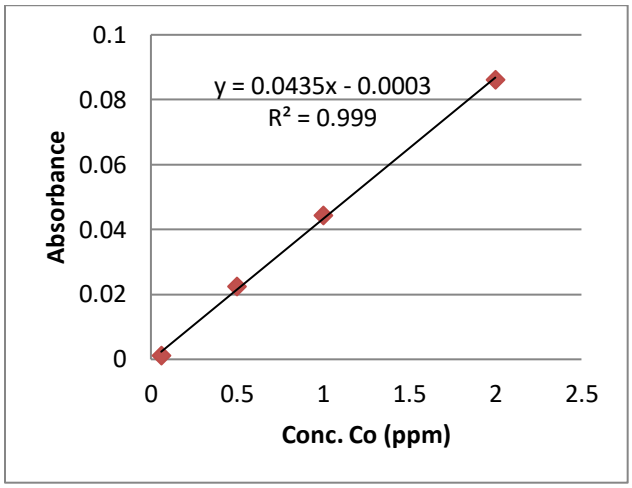


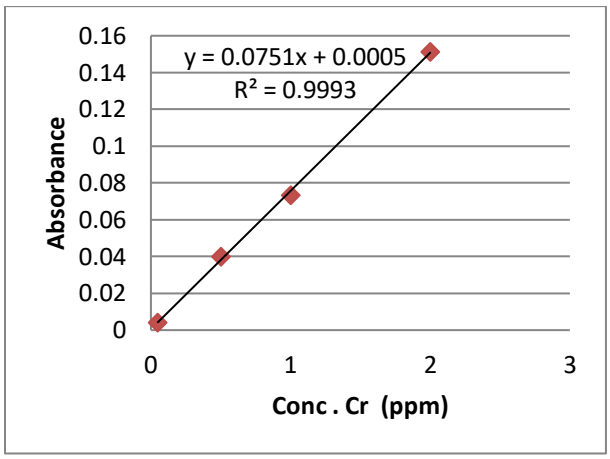


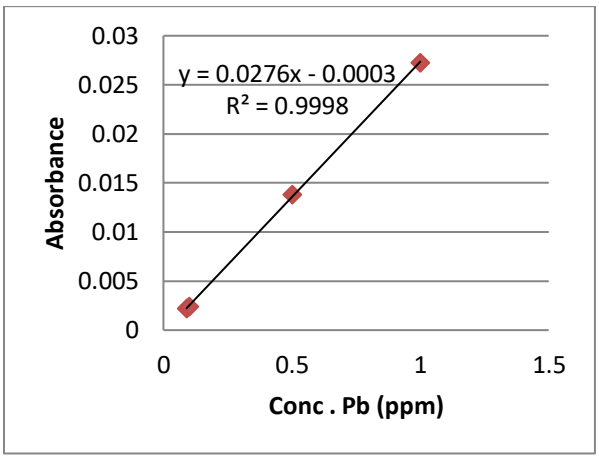





y = 0.027x + 0.0005
R² = 0.9993
Absorbance
Conc.Ni (ppm)
0.03
0.025
0.02
0.015
0.01
0.005
0
0
0.5
1
1.5


y = 0.0435x - 0.0003
R² = 0.999
Absorbance
Conc. Co (ppm)
0.1
0.08
0.06
0.04
0.02
0
0
0.5
1
1.5
2
2.5


y = 0.0751x + 0.0005
R² = 0.9993
Absorbance
Conc . Cr (ppm)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
1
2
3


y = 0.0276x - 0.0003
R² = 0.9998
Absorbance
Conc . Pb (ppm)
0.03
0.025
0.02
0.015
0.01
0.005
0
0
0.5
1
1.5

Appendix V: ANOVA analysis results for selected metals

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Calcium (mg/L)	Between Groups	2843561.270	6	473926.878	82308.459	.000
	Within Groups	322.444	56	5.758		
	Total	2843883.714	62			
Magnesium (mg/L))	Between Groups	16896.540	6	2816.090	871.812	.000
	Within Groups	180.889	56	3.230		
	Total	17077.429	62			
Manganese (mg/L)	Between Groups	.017	6	.003	14394.085	.000
	Within Groups	.000	56	.000		
	Total	.017	62			
Nickel (mg/L)	Between Groups	.000	3	.000	2952.799	.000
	Within Groups	.000	32	.000		
	Total	.000	35			
Copper (mg/L)	Between Groups	.015	6	.002	1562.193	.000
	Within Groups	.000	56	.000		
	Total	.015	62			
Cobalt (mg/L)	Between Groups	.004	6	.001	194.227	.000
	Within Groups	.000	56	.000		
	Total	.004	62			
Chromium (mg/L)	Between Groups	.005	6	.001	15356.008	.000
	Within Groups	.000	56	.000		
	Total	.005	62			