HAWASSA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

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DEPARTMENT OF CHEMISTRY

ASSESSMENT OF THE PHYSICOCHEMICAL QUALITY AND MINERAL COMPOSITIONS OF RAW COW'S MILK COLLECTED FROM MILK PRODUCER IN HAWASSA, SIDAMMA REGION, ETHIOPIA

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MARCH, 2025

HAWASSA, ETHIOPIA

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By: ASHENAFI LORENSO GIRO

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

ADVISOR: MELAKU ZIGDE (ASS. PROFESSOR)

MARCH, 2025

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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This MSc thesis has been submitted for examination with my approval as thesis advisor.

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LISTS OF ACRONYMS/ABBREVIATIONS

FAAS……………………Flame Atomic Absorption Spectroscopy

CODEX…………………Codex Aliment Arius Commission

ESA …………………….Ethiopian Standards Agency

FAO……………………..Food and Agricultural Organization

ISO………………………International Standard Organization

WHO……………………World Health Organization

EU……………………….European Union

MDL…………………….Method detection limits

IDL……………………...Instrumental detection limits

ANOVA………………...Analysis of Variance

LOD…………………......Limit of detection

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ABSTRACT

Milk is a wholesome food as it contains various nutrients. Milk should be free from contaminants and adulterant materials that can directly or indirectly affect the consumer's health. It is, therefore, necessary to check the safety and quality of milk available in the domestic market. Therefore, this study was aimed at determining the levels of selected physicochemical quality parameters and metals in raw cow's milk samples collected from milk producer, in Hawassa city, Sidamma Region, Ethiopia. The determination of fat, Solids-Non-Fat, milk density and added water to milk were carried out using EKOMILK Ultrasonic Milk Analyzer, in Hawassa University, and the total moisture and total ash content in raw cow milk samples were done using the Gravimetric Method. The values of total protein, total solids, and lactose content of the raw cow's milk samples were calculated using formulae that used the measured value of Solids-Non-Fat, while the metal content were determined by flame atomic absorption spectrometry (FAAS). The average values of the physicochemical parameters investigated in this study were found to be in the range pH (6.59–6.73), water added (5.54–10.33)%, density (1.023–1.029) g/mL, titratable acidity (0.153–0.213)%, total solids (10.566–13.800)%, total ash (0.603–0.636)%, Solids-not-fat (SNF) (7.266–7.666)%, total fat (3.266– 6.230)%, total protein (2.666–2.813)%, and lactose (3.996– 4.216)%. The average concentrations of the metals were found to be in the range Ca (1124.770–1505.110) mg/L, Mg (102.330–109.000) mg/L, Cu (0.0536–0.0817) mg/L and Mn (0.052–0.072). Metals such as Ni, Co, Cr, and Pb were found to be below the detection limit of the instrument. The accuracy of the method of the analysis was evaluated by analyzing the digest of the spiked samples and the recoveries of the metal were in the range of 99.810–104.220%. One-Way-ANOVA analysis showed that there were significant variations ($p < 0.05$) in the physicochemical quality parameters, and metal content except for total ash, solids-non-fat, total protein, and lactose among the raw cow's milk samples. In general, pH, specific gravity, titratable acidity, total ash, and lactose were found to be within the maximum permitted level set by Ethiopian Standard, and EU guidelines, while total fat, total protein, and solid-not-fat were found to be below the recommended limits.

Keywords: Raw milk, quality, physicochemical, metals, Hawassa

1 INTRODUCTION

1.1 Background of the study

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 [1,2]. 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 cow, buffalo, goat, sheep, reindeer and camel are utilized for their milk production [5, 6]. However, 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 [7, 8]. The major chemical components of milk include water, fats, carbohydrates, minerals, organic acids, enzymes and vitamins [9, 10]. However, 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, and weather [11, 12].

Compared with other foods, milk offers a cheap and valuable source of vitamins and minerals [13], and also contains a number of active compounds that play a significant role in both nutrition and health protection [14]. Fresh whole milk is considered as the complete food (diet) because it contains all the essential nutrients such as lactose, fat, proteins, minerals and vitamins in balanced ration rather than other food [15] and is definitely one of the most valuable and regularly consumed foods. But at the same time, it is highly vulnerable to bacterial contamination and its quality starts deteriorating during handling, processing and storage [16]. Hence it is easily perishable [17, 18]. Moreover, milk adulteration has recently become a major issue to be concerned all over the world [19]. Generally, raw milk is adulterated by using potable water or whey (watery part of milk remaining after making of cheese) which is known as economic adulteration and is commonly practiced by the supplier mainly to increase the quantity [20], while the other adulterants include skim milk powder, salt, detergents, cane sugar, urea, formalin, coloring agents, starch, and acids [21, 22]. Thus, to get rid from these problems, continuous monitoring the quality and adulteration of milk are so important. There are several modern and new techniques used to identify the presence of the adulterants in milk [20], in which the usual checking physicochemical parameters of knowing the milk's quality and adulterations include the measurement of pH, water added, density, total crude fat, total crude protein, titratable acidity, total solid (TS), solid-not-fat (SNF), lactose, total ash, and microbial count [23]. Therefore, this study was undertaken to determine selected physicochemical parameters and mineral composition of raw whole cow's milk sold from milk producer, marketing systems in which governments do not intervene substantially in marketing, in Hawassa city, Sidamma Region, Ethiopia. This informal market involves direct delivery of fresh milk by producers to consumers/traders/cooperatives in the immediate neighborhood or to any interested individuals in nearby towns [24].

1.2 Statement of the problem

Milk can easily be contaminated by many different sources including the udder and body of cows, dust from the air, litter, floor, flies, insects and rodents, water supply, hands and clothes of the milker, utensils, bottles, atmosphere etc. [25–27]. Thus, milk and the dairy products can be important sources of food borne pathogens [28]. Moreover, adulteration of milk with water, which is very common worldwide, not only causes dilution of milk reducing the milk solids, and further decreasing its quality but also involves the risk of introducing germs into the milk that might cause spreading infectious and contagious diseases [29, 30]. Therefore, based on these above concerns, this research was carried out to assess the physicochemical quality and mineral composition of the milk and to determine the presence of any added adulterants to the commercial raw milk sold informally in the local markets in Hawassa, Sidamma Region, Ethiopia.

1.3 Objectives of the study

1.3.1 General Objective

The main objective of this study was to determine selected physicochemical quality parameters and mineral composition of raw whole cow's milk collected from milk producer in Hawassa, Ethiopia.

1.3.2 Specific Objectives

- To determine the concentration of selected metals (Ca, Mg, Mn, Ni, Cu, Co, Cr, and Pb) in raw whole cow's milk in the study area.
- To compare the present study with national and international standards and with other similar studies.

1.4 Significance of the study

- The study provides insights into the quality of raw whole cow's milk collected out from milk producer in Hawassa, Ethiopia. The study also helps regulatory bodies to ensure the quality and safety of raw whole cow's milk sold out in local informal market in Hawassa, Ethiopia. Lastly, the findings of this study may help milk producers to improve the quality and safety of their products, and provides a basis for further research regarding milk production, safety and quality.

1.5 Limitations of the Study

The limitation of this study was the analysis of few raw whole cows' milk samples, and the exclusion of some of the physicochemical parameters due to lack of instruments, apparatus, and reagents in Hawassa University, chemistry laboratory.

2 LITERATURE REVIEW

2.1 Overview of dairy production in Ethiopia

Dairying is practiced almost all over Ethiopia involving a vast number of small or medium or large-sized, subsistence or market-oriented farms due to its large livestock population. According to the Central Statistical Agency (CSA) [31] the cattle population was estimated at about 56.7 million. From the total cattle population, 44.55% are males and 55.45% females. Despite such a substantial potential, the dairy sector is not developed to the expected level [32].

Based on climate, land holdings and integration with crop production as criterion, Ethiopia's dairy production systems can be broken down into three main systems: (1) the rural dairy system which is part of the subsistence farming system and includes pastoralists, agro-pastoralists, and mixed crop–livestock producers, (2) peri-urban, and (3) urban [32–34]. The rural dairy system (pastoralism, agropastoralism and highland mixed smallholder production system) produces the largest share of total milk produced, contributing 98% of the milk supply, while the peri-urban and urban dairy farms produce only 2% of the total milk production of the country [33, 35].

The rural dairy system is non-market oriented and most of the milk produced in this system is retained for home consumption. The surplus is mainly processed using traditional technologies and the processed milk products such as butter,cheese and sour milk are usually marketed to urban areas (primarily through informal market) when transport is available and affordable [36].

Urban and peri-urban dairy production systems involve production, processing and marketing of milk and milk products specifically targeting consumer in the nearby town and city [37, 38]. These market-oriented systems are emerging as important components of the milk production systems in Ethiopia. These systems are contributing immensely towards filling the gap between demand and supply for milk and dairy products in urban centers, where consumption of milk and milk products is remarkably high [37].

Peri-urban milk production is developed in areas where the population density is high and agricultural land is shrinking due to urbanization around big cities like Addis Ababa. It possesses animal types ranging from 50% crosses to high grade Friesian in small to medium-sized farms. The peri-urban milk system includes smallholder and commercial dairy farmers in the proximity of Addis Ababa and other regional towns. This sector owns most of the country's improved dairy stock [36]. The main source of feed is both home produced or purchased hay; and the primary objective is to get additional cash income from milk sale. This production system is now expanding in the highlands among mixed crop–livestock farmers, such as those found in Selale and Holetta, and serves as the major milk supplier to the urban market [39].

Urban dairy farming is a system involving highly specialized, state or businessmen owned farms, which are mainly concentrated in major cities of the country. They have no access to grazing land. Currently, a number of smallholder and commercial dairy farms are emerging mainly in the urban and peri-urban areas of the capital [37, 40] and most regional towns and districts [41].

Dairy products in Ethiopia are channeled to consumers through both formal and informal dairy marketing systems. The informal market involves direct delivery of fresh milk by producers to consumer in the immediate neighborhood or sale to itinerant traders or individuals in nearby towns. In the informal market, milk may pass from producers to consumers directly or through two or more market agents. The informal system is characterized by no licensing requirement to operate, low cost of operations, high producer price compared to formal market and no regulation of operations [42]. Hence, the informal (traditional) market has remained dominant in Ethiopia Production is non-market oriented and most of the milk produced is retained for home consumption.

2.2 Dairy production in Hawassa city

Urban and peri-urban dairying plays an important role in the Sidamma regional state, Ethiopia. Hawassa and Yirgalem, for instance, are among the major urban areas of the region where livestock farming is an important component of agriculture. These cities/towns are also among the high potential areas for milk production in Sidamma region [43].

The main feed resources available in the farm for livestock in Hawassa city were crop residues, including stover (especially, maize), grass hay, industrial by-products and to some extent Attela (a by-product of a local alcoholic beverage). Common salt as mineral supplement was offered to their dairy animals. Fresh grass, hay, and flour and oil industry by-products such as wheat bran, wheat middling and noug (Guizotia abyssinica) cake were also used as a feed for their animals. Occasionally, fruit byproducts (wastes of fruits and Vegetables, leftover food) can also be used as a feed for their animals. Animals were also let loose in the city in search of feed, and deep wells, tap water and rarely the Lake Hawassa and accessible rivers are drinking water sources for their animals [43].

2.3 Milk and its health benefits

Milk and dairy products are nutrient-dense foods, supplying energy and high-quality protein with a range of essential micronutrients (especially calcium, magnesium, potassium, zinc, and phosphorus) in an easily absorbed form [44–46]. Milk minerals are crucial for human health and development especially in childhood [44]. Adequate calcium intake influences skeletal calcium retention during growth and thus affects peak bone mass achieved in early adulthood [47, 48]. The high levels of calcium play an important role in the development, strength, and density of bones for children and in the prevention of bone loss and osteoporotic fractures in elderly people [48, 49, 50]. Calcium also has been shown to be beneficial in reducing cholesterol absorption, and in controlling body weight and blood pressure [49].

Dairy products are rich in nutrients that are essential for good bone health, including calcium, protein, vitamin A, vitamin B-12, vitamin D, riboflavin, potassium, phosphorus, selenium, magnesium, zinc, bioactive peptides and oligosaccharides [50, 51]. They mostly emerge during fermentation or digestive processes while in some cases these are components of fresh milk.

The dairy proteins play a vital role in food intake regulation, satiety and metabolic distracts relating to obesity. Blood pressure may be affected by lactic acid bacteria, milk proteins, peptides and calcium. Milk fat contains certain components having the functional significance. Antimicrobial effects are exerted by sphingolipids and their active metabolites either directly or upon their digestion. Whey proteins, i.e. α-lactalbumin, lactoferrin, lactoperoxidase, serum albumin and β-lactoglobulin acquire important biological and nutritional properties particularly regarding disease prevention. Immunostimulatory, anticarcinogenic and antimicrobial are other whey protein activities that promote health [51].

Milk has a wide various biologically active substances for instance, enzymes, immunoglobulin, oligosaccharides, antimicrobial peptides, hormones, cytokines, and the growth factors besides the basic saccharides, proteins and lipids [52]. Milk components are particular parts of immune system of newborn and they assist to stimulate and sustain the baby immune homeostasis.

2.4 Physicochemical quality of milk

Milk's physical composition was an important parameter, which indicates the nutritional qualities of milk [53]. Milk has unique physical properties, which are used as quality indicators. The principal physical properties of milk include its pH, specific gravity and density, water added, titratable acidity, proximate composition, which include fats, proteins, carbohydrate, and ash or minerals.

2.4.1 pH

pH is a measure of the solution's acidity or alkalinity. The pH of milk indicates whether the milk is acidic or alkaline and whether the milk is slightly acidic or close to neutral [54]. The normal pH of fresh milk from healthy cow's milk is in the range of 6.5–6.7. Lower pH values can occur due to the growth of microorganisms that ferment lactose into lactic acid or extension lipolysis whereas higher pH values in milk can occur during physiological stress of the animal when the mineral balance of the milk is altered by changes in the permeability of the blood barrier and milk (e.g. in the late stages of the lactation cycle or during mastitis infections) [55, 56]. Lactic strains can ferment lactose into a carboxylic acid, with a rise in acidity and a decrease in pH of fermented milk. Thus, pH play important role in determining the standard of fermented milk since it indicates the amount of acid present within the milk.

2.4.2 Water added

Milk is most frequently adulterated by the addition of water, which reduces its nutritional value and contaminates the milk, leading to health problems [57]. "Water added" refers to the intentional or unintentional addition of water to milk, which can reduce or even damage the obtained milk [58]. Some factors that lead to the addition of water to milk are the gap between food demand and supply, as well as the desire to obtain additional profit [59]. Milk that has been diluted with water will impact the contents within the milk, resulting in a reduction of fat, protein, and other components [60]. Checking for "water added" in milk is necessary before using the milk for the cheese making process. The higher the "water added" content in the milk, the lower the purity, which will affect the texture of the cheese and can even lead to cheese damage. "Water added" in milk will change the specific gravity of the milk and alter the natural color of the milk.

2.4.3 Specific gravity or density

Specific gravity (Sp. Gr.) is the ratio of the density of a substance to the density of standard substance (water), both being at the same temperature. Thus, specific gravity of milk is the ratio of the weight of equal volumes of milk and distilled water at the same temperature, and it varies according to the proportion of fat, SNF (solid- not fat) and water. The specific gravity of the whole cow's milk ranges from (1.027 to 1.035) g/mL, with an average of (1.032) g/mL at 16 °C which means that milk is (1.032) times heavier than waters [61]. Addition of water or other substances changes the density. Addition of water reduces the density, while addition of solids increases the density considerably. If density is outside the normal range, it means the milk has been adulterated. The density of milk, among others, is usually used for quality test mainly to check for addition of water to milk or removal of cream. Addition of water to milk minimizes milk density, while removal of cream increases it [61].

2.4.4 Titratable acidity

The titratable acidity (TA) provides an indirect measure of the acidity (Natural + developed) content in milk. All milk has a natural acid content attributed to proteins, minerals and dissolved gasses. However, when milk acid content is increased by the bacteria that convert lactose to lactic acid, the acidity is known as developed or real titratable acidity [61, 62]. When this occurs, a dramatic increase in TA value is observed. Titratable acidity is expressed in terms of percentage lactic acid since lactic acid is the principal acid produced by fermentation after milk is drawn from the udder [63]. Fresh milk, however, does not contain any appreciable amount of lactic acid high quality raw milk has a relatively steady TA value ranging between 0.14 to 0.17% (expressed as lactic acid). The degree of bacterial contamination and the temperature at which the milk is kept are the main factors influencing acid formation. Therefore an increase in acidity is a rough measure of its age and bacterial activity [64].

2.4.5 Total ash

Ash content in foodstuffs indicates the presence of inorganic minerals in the foodstuffs. Ash level is a reflection of mineral contents in a sample [65]. 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.

2.4.6 Total fat

Raw milk is usually composed of about 4% fat, 3.2% proteins, 4.6% lactose and a number of micronutrients [66]. 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 milk fat globules (MFG) and has a hydrophobic character [67]. 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 mono-acyl glycerol, cholesterol and cholesterol esters, free fatty acids, and phospholipids are present [68]. The size of the MFG varies from 0.1–20 μm [69].

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 [70]. 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 linoleic 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 [71].

2.4.7 Crude protein

Protein has a large function in human body (For example, building the structural organs) and in fact, the human body is about 45% proteins. It is an essential macromolecules 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 [72]. Protein also aid in the formation of antibodies that enable the body to fight infections. Protein serves as a major energy supplier [73]. The building blocks of protein are Amino acids. Proteins are therefore polymers of amino acids, most of which are α-amino acids, having 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.

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-lacto globulin, α-lacto globulin, serum albumin and immunoglobulin. The distinction between the two groups is based on solubility at pH 4.6 [74].

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 cheese making process [75].

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-lacto globulin and α-lacto globulin, which are both synthesized by the secretory cells. β-lacto globulin makes up the major part of the whey proteins, α-lacto globulin, as previously mentioned, is a coenzyme in the synthesis of lactose. Other major whey proteins include the immunoglobulin and IgG, IgA and IgM occur at high levels in colostrum with the biological function to provide protection to the newly born calf [75].

2.4.8 Total solids and Solids–not-fat

The total solids (TS) content is a measure of the amount of material remaining after all the water has been evaporated. Solids–not-fat (SNF) portion consists of protein (primarily casein and lacto globulin), 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.

2.4.9 Carbohydrate/Lactose

Lactose (milk sugar) is the principle carbohydrate and a major component in milk. It is a naturally occurring sugar that is almost exclusively found in mammalian milk [76, 77]. 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 [78]. Lactose is a reducing sugar [78] 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 [79], and gives milk its sweet taste, although lactose has low relative sweetness.

2.5 Minerals in milk

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 [80], 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 ppm) arsenic, copper, iron, nickel, tin, chromium, fluorine, manganese, selenium, vanadium, cobalt, iodine, molybdenum, silicon and zinc; (III) Non-essential toxic mineral elements beryllium, lead, cadmium, palladium, mercury and thallium. 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 [81]. Potassium aids in the control of electrolytes, water, acid-base balance, and muscular function in the body [82]. 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 [82, 184].

2.6 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 [85, 86]. 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 [87, 88]. Moreover, the essential trace element profile of milk, particularly toxic element residues, is largely affected by the environment where the cows are raised [86, 88, 89].

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 [85, 86, 88, 90–92]. 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 [86] 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 [87, 88] 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 the 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 monitor regularly milk quality, paying special attention to toxic metals. Theirs concentration in milk, especially in industrial regions, may serve as a direct bio indicator of the quality milk and its products, but can also be an indirect indicator of contamination in the environment where milk is produced [93, 94].

2.7 Milk quality standards

The overall quality of food, including milk and milk products, is closely tied to various factors such as its physical (pH, specific gravity, temperature, titratable acidity), chemical (fat, protein, lactose, minerals, vitamins, water) , and microbiological (total bacteria count, yeast and mould count, lactic acid bacteria count, total coliform count) characteristics. Therefore, it is fundamental to conduct the physicochemical and microbiological studies to monitor and assess the quality of food [95–97].

The quality assessment of food such as milk and milk products are intended to confirm that the milk and milk products fit the accepted standards for nutritional composition, cleanliness and levels of a variety of microorganisms [98]. There are methods and standards for the determination of the quality parameters for milk and milk products that has been recognized by national and international organizations. In Ethiopia, there are mandatory and voluntary standards related to dairy production and processing. These include voluntary standards such as ES 3460:2009 (unprocessed whole/raw cow milk), ES 3468:2009 (yogurt), ES 3466:2009 (cream), and ES 3462:2009 (pasteurized liquid milk), and Compulsory Ethiopian Standards (CES) standard such as CES 278 : 2021 raw whole cow milk – specification, CES 279 : 2021 pasteurized liquid milk-specification, and CES 281 : 2021 UHT milk – specification.

The quality criteria that can be used for the determination of unprocessed whole/raw cow milk quality according to Ethiopian Standards and European Union (EU) Standards is summarized in Table 2.1.

According to the Ethiopian Standards, these quality standards are not compulsory and can be voluntarily agreed upon, while according to the EU standards and Compulsory Ethiopian Standards (CES), they have to be fulfilled by all commercial producers, distributor or any other concerned body.

Table 2.1: Unprocessed whole/raw cow milk specifications

Nutrients (%)	**Ethiopian Standard (ES 3460:2009)[99]**	**Ethiopian standard (CES 278: 2021) [100]**	**European Union (EU) quality standards (Raff, 2011) [101]**
Fat	> 3.5%	3.34 Min.	3.25
Protein	> 3.2%	3.07 Min.	2.73
Total solids	> 12.8%	12.80	12.5
SNF	NA	8.38Min.	8.25
Lactose	NA	NA	4.2
Density at 15.6℃	1.023–1.032		
TA(g/l)	0.2Max.		
Total ash		NA	> 0.69%

NA: Not Available

3 MATERIALS AND METHODS

3.1 Description of the study area

The study was conducted in Hawassa city, Sidamma Regional State (Figure 3.1). Hawassa, the regional capital of Sidamma Regional State, is found 275 km south of the capital Addis Ababa along Addis Ababa–Moyale highway. It has an altitude of 1750 m asl, and is located at $6^o83'$ to $7^o17'$ N and 38^o 24' to $38^o72'$ E (Figure 1). Hawassa receives an average annual rainfall of 955 mm with mean annual temperature of 20 °C [102], and the city has a total area of about 50 km^2 divided into eight sub-cities and 32 kebeles (kebeles are the smallest administrative unit below the sub-city/woreda level).

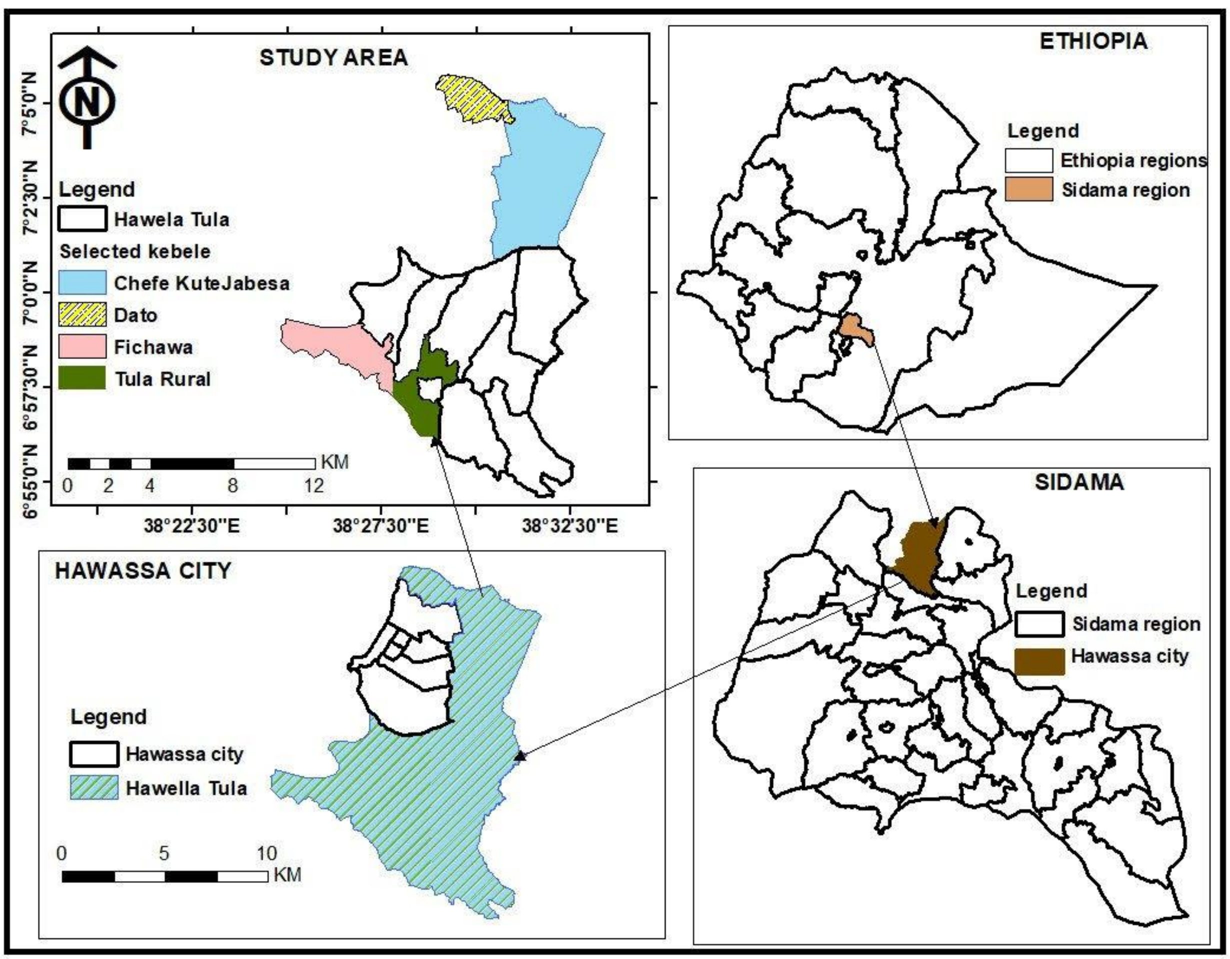

Figure 3.1 Location of the study area

3.2 Sampling and sample collection

Tula sub-city has the highest milk production in the city. The selected sub-city was Tula sub-city that has twelve kebeles. Out of twelve kebeles, four kebeles (Dato, Chefekotijebesa, Finchawa, and Tula Geter) were selected purposefully as these kebeles were the major milk producing kebeles in Tula sub-city having at least 60–70 milking cows. Then, one commercial dairy farm found in each kebele was selected randomly, and the raw whole milk sample was purchased from milk selling shops of each dairy farm respectively. Thus, a total of four raw whole milk sampling sites were included in this study. A total of 2.0 liters of raw whole cow milk were collected during August 2024 and transported in cooled box with icepack into the Hawassa University laboratory and Chilled at 4 °C before immediate analysis. The determination of fat, Solids-Non-Fat, milk density and added water to milk were carried out using EKOMILK Ultrasonic Milk Analyzer, in Hawassa University Dairy processing plant. The rest of the physicochemical parameters were determined in Chemistry laboratory, Hawassa University.

3.3 Chemicals and Standards

A 65% nitric acid (HNO_3) and 30% hydrogen peroxide (H_2O_2) were used for the digestion of the pasteurized cow milk samples. Distilled water was used for all dilutions. Sodium hyroxide (NaOH, 0.1 M) and phenolphthalein were used for the determination of titratable acidity; Stock standard solutions of concentration 1000 mg/L of the metals Ca, Mg, Cu, Mn, Co, Cr, Ni, and Pb (BUCK SCIENTIFIC, PURO-GRAPHIC) as nitrate salt standard solutions were used to prepare intermediate standard solutions.

3.4 Apparatus and Instrument

The apparatus that was used during the study included: 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 raw whole cow milk samples, spiked whole cow milk samples, and blank solutions. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the collected samples and digested samples until analysis. Portable pH meter (PH/ORP/Temp Meter), A digital analytical balance (Mettler Toledo, Model AG250, and Switzerland with ± 0.0001 g) was used to weigh raw whole cow milk samples and reagents. EKOMILK Ultrasonic Milk Analyzer (MILKANA KAM98 – 2A) 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 raw whole cow milk samples for the selected elemental analysis.

3.5 Preparation of samples for mineral analysis

Many researchers use microwave digestion techniques to solubilize biological samples [103, 104], 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 using nitric acid (HNO_3), 65% (w/v) supra-pure (Merck) and hydrogen peroxide (H_2O_2), 30% (w/v) [105]. These 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.

3.5.1 Optimization of acid 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 [106]. 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 digestion condition for a fixed amount of raw whole cow milk sample were selected based on minimum volume of acids, digestion time, and digestion temperature and clear digest solution. The different conditions taken and tried are shown in the appendix III.

3.5.2 Optimized acid digestion procedure

The digestion of raw whole cow milk samples were carried out after optimization of the digestion procedures Applying the optimized conditions (i.e., 4.0 mL HNO_3 (65%): 2.0 mL H_2O_2 (30%) volume ratio of reagents, 180 °C digestion temperature and 1:30 hours digestion time) for the digestion of 5.0 mL raw cow's milk samples), 5.0 mL of raw cow's milk sample was transferred into a 250 mL round bottom flask, and a mixture of HNO_3 (65%) and H_2O_2 (30%) with a volume ratio of 2:1 (v/v) was added and the mixture was digested on a Kjeldahl digestion apparatus fitted with a reflux condenser by setting the temperature at 180 °C and digested for 1:30 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 (What man 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.

3.6 Determination of the physicochemical quality of raw whole cow milk samples

The determination of fat, Solids-Non-Fat, milk density and added water to milk were carried out using EKOMILK Ultrasonic Milk Analyzer, in Hawassa University Dairy processing plant. The determination of total solids content, lactose content, and total protein content of the milk were determined by using the following formulae [107],

$$Lactose\ content\ = SNF \times 0.55\%$$

$$Total\ solids\ = 100\% - \%\ Moisture$$

$$Total\ protein\ = SNF \times 0.367\%$$

3.6.1 Determination of pH

The pH of raw whole cow milk samples were determined using portable pH meter, after the pH meter was calibrated with standard buffers solution pH= 4.0 and pH=7.0. Then, 5.0 mL of raw whole 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 raw whole cow milk samples.

3.6.2 Determination of Titratable acidity

Titratable acidity of the raw whole cow milk samples was expressed as percent lactic acid [108]. 5.0 mL raw whole cow 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 0.1N NaOH solution until a faint pink color appears. The volume of NaOH consumed during the titration was recorded and used to calculate the Titratable acidity (%) as follows:

$$\text{Titratable acidity(as \% lactic acid)} = \frac{(Volume\ of\ 0.1M\ NaOH\ (mL) \times 0.009)}{Weight\ of\ raw\ cow\ milk\ sample\ (g)} \times 100$$

3.6.3 Determination of Moisture

The determination of water content was carried out using the Gravimetric method proposed by AOAC [109] and it was performed in triplicate. Two grams of milk sample were measured and put in the oven for 5 hours at 110 °C. Then it was cooled in a desiccator for 10 minutes and weighed. The sample was put back into the oven for 30 minutes, cooled in a desiccator, and weighed. The treatment was performed several times until it reached a constant weight, and the water content was calculated by the formula:

$$Water\ content\ (\%) = \frac{(Weight\ of\ fresh\ sample - Weight\ of\ dry\ sample)}{Weight\ of\ fresh\ sample} \times 100$$

3.6.4 Determination of total ash

Determination of ash content (mineral contents) in raw whole cow milk was done using the Gravimetric Method [109]. The dried milk samples used for determination of total solids content were ignited in a muffle furnace at a temperature of 550 °C for 3.00 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:

$$Total\ Ash\ (\%) = \frac{(Weight\ of\ residue)}{Weight\ of\ sample} \times 100$$

3.6.5 Determination of Minerals

The mineral contents of the raw whole cow milk samples were determined by atomic absorption spectrometry according to the methods of AOAC [110]. The digested raw whole cow milk samples were analyzed to determine the concentration of Ca, Mg, Mn, Ni, Cu, 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.

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 raw whole cow 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.

The working standard solutions for each metal were prepared from Stock standard solutions (1,000 ppm) of each element. The dilution factor for all minerals except Ca and Mg was 10. For 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 50 mL. The concentrations of elements recorded were in terms of ppm.

3.7 Instrument operating conditions and Calibration

3.7.1 Instrument operating conditions of FAAS

For the determination of elements such as Ca, Mg, Mn, Ni, Cu, Co, Cr, and Pb were carried out 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 3.1 showed the instrumental conditions for each element analyzed.

Table 3.1: FAAS instrumental operating conditions for the determination metals

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

3.7.2 Calibration of AAS

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 3.2.

Table 3.2: Concentrations of working standards for each metal analyzed using FAAS

Ca	**Mg**	**Mn**	**Ni**
0.5; 1.0; 2.0; 4.0	0.05; 2.0; 4.0; 6.0	0.05; 0.1; 0.3; 0.5	0.06; 0.1;0.5; 1
Cu	Co	Cr	Pb
0.008; 0.5; 1.5; 2.0	0.06; 0.5; 1; 2	0.05; 0.5; 1; 2	0.09; 0.1; 0.5; 1.0

3.8 Method validation

3.8.1 The method detection limit (MDL)

The method detection limits for each metal were estimated using blank digest for selected raw whole cow 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 [111], MDL =3×Standard deviation of the blank

3.8.2 Recovery Test

As there were no certified standard reference materials 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 (%) [112]. A spiking experiment was done to evaluate or check the accuracy of the digestion procedure and the atomic absorption spectrophotometer used for metal analysis. In this experiment, one sample was selected (5.0 mL of raw whole cow milk sample), spiked with 50 μL of 1000 mg/L of Ca, Mg, Cu, and Mn standard solutions at once and digested following the same digestion procedure used for unspiked raw cow's milk samples. The spiked samples were digested 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:

$$\% \, Recovery = \frac{Amount \; recovered}{Spiked \; amount} \times 100$$

$$= \frac{(Conc. spiked \; sample \; - \; Conc. unspiked \; sample)}{Spiked \; amount} \times 100$$

3.9 Statistical Analysis or Data Analysis

The data obtained from the chemical analysis of the samples was analyzed using descriptive statistics employing SPSS version 16.0, and Microsoft excel program. Common descriptive statistics such as mean, standard deviation, and % RSD were used to describe the data while one-way analysis of variance (ANOVA) was applied to examine statistical significance differences among the mean values of the physicochemical parameters and mineral compositions of the different raw whole 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 Physicochemical composition of raw cow's milk samples

The physicochemical properties of raw cow milk samples collected from four sampling sites in the study area were shown in Table 4.1.

Table 4.1 Physicochemical analysis results (Mean ± SD, n=3, %) of raw cow milk samples

Parameters				
	Sample-1	Sample-2	Sample-3	Sample-4
pH	6.733± 0.015	6.623± 0.015	6.590± 0.010	6.630± 0.026
Water added (%)	10.333± 0.305	5.540± 0.314	6.366± 0. 0152	6.086± 0.1026
Density (g/mL)	1.023± 0. 001	1.027± 0. 001	1.024± 0. 001	1.029± 0. 001
Titratable acidity (% lactic acid)	0.153 ± 0.006	0.186 ± 0.006	0.213 ± 0.006	0.166 ± 0.006
Moisture (%)	86.200± 0.100	88.030± 0.150	89.430± 0.550	89.266± 0.057
Total solids (%)	13.800± 0.100	11.966± 0.150	10.566± 0.550	10.730± 0.058
Total ash (%)	0.628 ± 0.021	0.636 ± 0.017	0.603 ± 0.042	0.620± 0.013
Solid-not-fat (%)	7.566± 0.252	7.666± 0.208	7.266± 0.503	7.466± 0.153
Total fat (%)	6.230± 0.153	4.166± 0.153	3.300± 0.100	3.266± 0.115
Total protein (%)	2.777± 0.092	2.813± 0. 076	2.666± 0. 185	2.740± 0.056
Lactose (%)	4.160± 0.138	4.216± 0.114	3.996± 0.276	4.106± 0.084

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 raw cow milk samples ranged from 6.59 to 6.73 as given in Table 4.1. The highest mean value (6.73) of pH was recorded in sample 1, while the least mean value (6.59) of pH was recorded in sample 3 (Table 4.1). One-Way ANOVA analysis showed that the pH content was significantly different, $F(3, 8) = 36.377$, $P < 0.001$, among the four raw cow milk samples. However, Post hoc test revealed the pH value of sample 2, 3, and 4 were not statistically significantly different. The results of ANOVA analysis for each physicochemical parameter were shown in appendix II.

According to the Ethiopian Standard for raw whole cow milk specification [99], the pH of raw whole cow milk ranges from 6.6 to 6.8, and the results showed that the mean pH value of all raw cow milk samples were within the ES pH range indicating that the milk samples were collected shortly after milking. A pH lower than the minimum set value of the ES 6.6 to 6.8, indicating prevalence of bacteria growth and multiplication, and above 6.8 indicating udder infection (mastitis) [113]. The present results were higher pH value than reported by [114] in milk from farms, cooperative and market 6.39, 6.32 and 6.195 respectively; and a study conducted in milk shades around Addis Ababa, Ethiopia [115]. These might be due to poor milk handling and storing practices.

Water added (%)

The average water added (not water content, only added water) content of raw cow milk samples analyzed were varied from 5.54% to 10.33%. The highest value was in sample 1 and the lowest value was in sample 2 (Table 4.1). One-Way ANOVA analysis showed that there was a significant difference among the four raw cow milk samples on the values of water added (%), $F(3, 8) = 284.895$, $P < 0.001$. Post hoc test revealed the water added (%) in sample 1 was significantly different from the other three samples. However, the water added in sample 2 and sample 4 and sample 3 and sample 4 were not significantly different.

The present study showed that all raw cow milk samples were diluted with water, and the results were higher than Haftu K S., et al., [116] who reported overall mean value of added water in milk producers (S1) and cafeteria (S2) samples in Hossana city of Hadiya zone were sample S2

(2.30%) and sample S1 (1.08%) respectively. A similar study conducted in three regions of Ethiopia found that water adulteration was prevalent along the value chains, with rates ranging from 1.82% to 10.4% [117]. Additionally, a study conducted in Selale and around Addis Ababa City reported that Milk samples were adulterated with water up to 33.9% [53].

Generally, water adulteration of milk (addition of water to milk) is done to increase the volume of milk and to make it less expensive or to gain double profits [118]. However, consuming milk that has been diluted with water can reduce its nutritional value and increase the risk of food poisoning through the introduction of various disease causing microorganisms, harmful chemicals and poisonous substances and become a health risk to the consumer [119, 120].

Specific gravity or density (g/mL)

The average density of raw cow milk samples analyzed was varied from 1.02270 g/mL to 1.02867 g/mL (Table 4.1). The highest density was in sample 4 and the lowest density was in sample 1. One-Way ANOVA analysis showed that there was a significant difference among the four raw cow milk samples on the values of density, $F (3, 8) = 89.086$, $P < 0.001$. However, Post hoc test revealed the density of sample 1 and 3 were not significantly different.

The normal density of raw milk depends on its composition and temperature can usually found in the range of 1.026 g/mL–1.032 g/mL at 20°C [121]. For normal whole cow milk, the specific gravity ranges from 1.023–1.032 at 15.6 ℃ compared to water (1.000 g/mL) based on the Ethiopian Standards (ES) [99]. Moreover, O'Connor [122] suggested that lower value of specific gravity than normal value of specific gravity of milk (1.020) is indicative of adulteration of milk with water which contributes to production of poor quality milk [123]. Similarly, specific gravity above the recommended level (1.035) indicates adulteration of milk by partial skimming. Based on this, the density of all the raw cow milk samples were within the normal density limit of milk (1.023 g/mL–1.032 g/mL) as regulated by Ethiopian Standards, 2009. This result is similar with Desye, et al., [115] who reported a specific gravity of 1.026 for raw cow milk samples in Gondar city, Northwest Ethiopia. In general, addition of water and removal of fat decreases the density of milk, while addition of solids such as flour or sugar increases the density of milk. Thus, the density measurement of milk quickly indicates nonconformities from the normal milk composition due to addition of water.

Titratable acidity (%)

Titratable acidity (also known as total acidity) was a measure of both bound and free hydrogen ions in solution. It measures the freshness, bacterial activity, and taste of milk and acknowledged as an indicator of milk quality. Milk with high or low titratable acidity levels can alter its taste and texture, as well as make it unsafe to consume [124].

In this study, the average titratable acidity ranges from 0.153%–0.213 % (Table 4.1). The highest titratable acidity value was recorded for sample 3 and the lowest was for sample 1. One-Way ANOVA analysis showed that there was significant difference among the four raw cow milk samples on the values of titratable acidity (%), $F (3, 8) = 61.333$, $P < 0.001$. However, Post hoc test revealed that the titratable acidity of sample 1 and 4 were not significantly different.

The titratable acidity values for all the four raw milk samples were reasonably lowered than reported acidity values for raw cow milk samples [124–126, 115]. However, the titratable acidity value of one raw cow milk sample was slightly higher than the acidity limit specified in Compulsory Ethiopian Standard /CES/ 278 2021 for raw Cow milk [100], which might suggest improper raw milk handling and storing practices that resulted in an increased bacterial growth, which in turn increased the titratable acidity. Therefore, it is important to properly handle and store raw cow milk to retain the quality and safety of the milk.

Total solid (%)

Total solids (TS) represent the total amount of non-water components in milk. The average total solid content of raw cow milk samples analyzed was varied from 10.566% to 13.80% (Table 4.1). The highest value was recorded for sample 1 and the lowest was for sample 3. One-Way ANOVA analysis showed that there was a significant difference among the four raw cow milk samples on the values of total solid content (%), $F (3, 8) = 78.614$, $P < 0.001$. However, Post hoc test revealed the total solid content (%) of samples 3 and 4 were not significantly different.

According to Ethiopian standards for the total solids content of cow milk is not to be less than 12.80% [99, 116]. Therefore, in this study three raw cow milk samples were found to have an average total solid content that were below the recommended standards, and were in agreement with reported total solids values for raw cow milk samples [117, 126]. This might be due to the practice of adulteration of milk by water or other solid materials, 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 [113].

Total ash content (%)

Total ash represents the total mineral content of milk. The average amount of total ash in raw cow milk samples analyzed were ranged 0.603% to 0.636% (Table 4.1). The highest value was in sample 2 and the lowest was in sample 3. One-Way ANOVA analysis showed that there was no significant difference among the four raw cow milk samples on the values of total ash content (%), $F(3, 8) = 0.913$, $P = 0.477$.

The average total ash content in all the raw cow milk samples analyzed were slightly less than the EU standard [101] for the ash contents of raw cow milk (0.69%). However, the ash content of the raw cow milk samples analyzed were within the usual range, 0.6% to 0.9%, of the ash content of the raw cow milk [127]. A similar study reported a high average ash content of 0.74% for raw cow milk collected from different value chain actors in Shashemene Town, Oromia region [114]. These variations in the ash contents of raw cow milk samples could be influenced by many factors such as feed quality, stage of lactation, or animal health [128, 129].

Solid-not-fat (%)

Solids-not-fat (SNF) is a measure of the nonfat components of milk. The average solid-not-fat (%) in raw cow milk samples analyzed was in the range of 7.266% to 7.666% (Table 4.1). The highest value was in sample 2 and the lowest value was in sample 3. One-Way ANOVA analysis showed that there was no significant difference among the four raw cow milk samples on the values of solid-not-fat content (%), $F(3, 8) = 0.913$, $P = 0.477$.

According to European Union (EU) quality standards, a minimum solid not fat (SNF) content of completely fresh milk is 8.25%. Similarly, the SNF content of raw cow milk should be 8.38% minimum, according to the Compulsory Ethiopian standard [100]. Therefore, the mean SNF content of all raw cow milk samples analyzed were lower than recommended standard SNF values, and were lower than reported SNF values for raw cow milk samples 8.7% [130, 131] and 8.90% by Gemechu et al., [126]. The low SNF content of raw milk samples might be due to the practice of adulteration of milk by water or other solid materials and other factors such as breed, feeding practices, or environmental conditions [128, 132].

Total fat (%)

The fat content of raw milk indicates its energy value [44], and the average total fat (%) content of raw cow milk samples analyzed were in the range of 3.266% to 6.23% (Table 4.1). The highest value was in sample 1 and the lowest was in sample 4. One-Way ANOVA analysis showed that there was significant difference among the four raw cow milk samples on the values of the total fat content (%), $F (3, 8) = 331.984$, $P < 0.001$. However, Post hoc test revealed the total fat content (%) of samples 3 and 4 were not significantly different.

In this study, two raw cow milk samples had milk fat content higher than that (3.50%) indicated in the Quality Standard Authority of Ethiopian [99], while the other two raw cow milk samples had milk fat content lower than that (3.50%) indicated in the Quality Standard Authority of Ethiopian as reported by Eshetu, et al. [133]. This low fat content of milk might be due to possible adulteration of milk by fat removal. The other reason could be due to the difference in feeding, management practices, season and breed of the animals [134].

The total fat content of one of the milk sample (6.23%) analyzed was comparable with a study by G. Dehninet, et al., [135] that revealed higher average fat content of 5.22% from milk samples collected from smallholder producers in Oromia and Amhara regions of Ethiopia.

Total protein (%)

The average total protein contents of raw cow milk samples analyzed were in the range of 2.666% to 2.813% (Table 4.1). The highest value was in sample 2 and the lowest value was in sample 3. One-Way ANOVA analysis showed that there were no significant difference among the four raw cow milk samples on total protein content (%), $F (3, 8) = 0.913$, $P = 0.477$.

The average protein content observed for all raw cow milk samples were below the Ethiopian standards [99] for protein content of fresh cows‟ milk (3.20%), and below the recommendation of the European Union quality standards for unprocessed whole milk total protein content should not be less than 2.9% [136]. In this study, the protein contents of all milk samples were lower than reported results 3.48% [137], 3.31% [138], and 3.46% [139] for milk samples collected from various milk supply chains in Dire Dawa, Ethiopia.

The variations in protein content for all raw cow milk samples might suggests the presence of under deficiency of crude protein in feeding concentrates, or due to factors such as breeds/genetics, health/physiology and environmental/management that influence protein levels in milk production [128, 132].

Lactose (%)

The average Lactose (%) content in raw cow milk samples analyzed was in the range of 3.996% to 4.216% (Table 4.1). The highest value was in sample 2 and the lowest value was in sample 3. One-Way ANOVA analysis showed that there were no significant difference among the four raw cow milk samples on the values of Lactose content (%), $F (3, 8) = 0.913$, $P = 0.477$.

According to the European Union Quality standards for unprocessed whole milk the lactose content should not be less than 4.2% [136]. Conversely, O'connor, [113] recommended the normal range of cow milk lactose content as 4.7% to 4.9%. Therefore, the mean lactose (%) content of all raw cow milk samples analyzed was close to the recommended EU standard [101]. These lactose values were lower than reported values (4.34%) [140], (4.76%) [117], and 5.08% [141]

4.2 Results of optimization of the acid digestion procedure

The experimental variables that were attempted during optimization of the digestion of exactly 5.0 mL of raw cow milk samples were shown in appendix III. Optimized procedures were selected based on the usage of lesser 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 raw cow milk samples in this study were (4.0 mL HNO_3 (65%): 2.0 mL H_2O_2 (30%) volume ratio of reagents, 180 °C digestion temperature and 1:30 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 analysed 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 analysed were shown in appendix II.

Table 4.2: Calibration graph equation and R^2 value

Metal	**Calibration Equation**	**R^2 Value**
Ca	Y= 0.0503x - 0.0019	0.9998
Mg	Y = 0.0016x + 0.0001	0.9995
Cu	Y = 0.0518x + 0.0013	0.9997
Mn	Y = 0.1127x - 0.0007	0.9996
Ni	Y = 0.0269x + 0.0005	0.9993
Co	Y = 0.0435x - 0.0003	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 analysed in raw cow's milk samples were shown in Table 4.3. These values were above the detection limit of the instrument.

Table 4.3: 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	0.008	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 metal contents of raw milk samples

The results of the concentration of the elements analyzed for each type of raw cow's milk samples were presented in Tables 4.4

The results of the concentration of metals in raw cow milk samples were presented in Tables 4.4 Metals such as Ca, Mg, Mn, and Cu were present in all raw milk samples while the concentration of Ni, Co, Cr, and Pb were found to be below the detection limit for all raw cow milk samples. In a similar study, heavy metals such as Co, Ni, and Pb in fresh cow's milk samples collected from four dairy farms were not detected [142]. However, various researches on mineral content in cow's milk were reported higher levels of these metals [143–149].

Table 4.4 Metal concentration (Mean ± SD, n=9, mg/L) of raw cow's milk samples collected from four sampling sites in Hawassa, Ethiopia

Parameters	Sample-1	Sample-2	Sample-3	Sample-4
Calcium	1124.777 ± 2.77	1140.330 ± 1.80	1505.11 ± 1.76	1137.666 ± 1.41
Magnesium	103.550 ± 1.94	108.330 ± 1.50	109.000 ± 1.73	102.33 ± 2.06
Copper	0.082 ± 0.002	0.061 ± 0.003	0.054 ± 0.002	0.076 ± 0.001
Manganese	0.052 ± 0.001	0.071 ± 0.001	0.072 ± 0.002	0.062± 0.002

Calcium

The mean calcium concentration in the analyzed raw cow milk samples ranged from 1124.77 to 1505.11 mg/L (Table 4.4). The highest mean calcium content was found in sample 3 and the lowest mean calcium content was found in sample 1. One-Way ANOVA analysis showed that there was a significant difference in the calcium content, $F(3, 32) = 77196.999$, $P < 0.001$, among the raw cow milk samples. The results of ANOVA analysis for the selected metals were shown in appendix IV.

Calcium plays a significant role, particularly in the developmental process of children. Approximately 75% of the daily calcium requirement is obtained from milk and dairy products [150]. The results of the calcium content of the raw cow's milk samples were higher than the results reported by Neslihan, et al., [151], as 118.10–433.45 mg/L mg/L for raw milk samples, Sikiric, et al., [152] and Malbe, et al., [153]. However, various researches on mineral content in cow's milk have indicated calcium levels ranging from 900 to 1200 mg/L [154–156].

Magnesium

The mean magnesium concentration in the analyzed raw cow milk samples ranged from 102.33 to 109.00 mg/L (Table 4.4). The highest mean magnesium content was found in sample 3 and the lowest mean magnesium content was found in sample 4. One-Way ANOVA analysis showed that there was a significant difference in the magnesium content, $F(3, 32) = 30.469$, $P < 0.001$, among the raw cow milk samples. However, Post hoc testing revealed that the mean magnesium concentration obtained for milk sample 2 and milk sample 3 was statistically similar. Similarly, Post hoc testing revealed that the mean magnesium concentration obtained for milk sample 1 and milk sample 4 was statistically similar.

Magnesium is a mineral that supports bone and heart health and regulates nervous system and muscle functions [157]. The results of the magnesium content of the raw cow's milk samples were lower than the results reported by Neslihan, et al., [151], as 197.81-294.19 mg/L mg/L for raw milk samples, Tizhooshet, et al., [157], and Elbagermi, et al., [158]. However, the results were almost similar to the magnesium concentration reported for raw cow's milk numbered 3-C [156].

Copper

The mean copper concentration in the analyzed raw cow milk samples ranged from 0.0536 to 0.0817 mg/L (Table 4.4). The highest mean copper content were found in sample 1 and the lowest mean copper content was found in sample 3. One-Way ANOVA analysis showed that there was a significant difference in the copper content, $F(3, 32) = 570.108$, $P < 0.001$, among the raw cow milk samples.

The results of the copper content of the raw cow's milk samples were far below the results reported by Hasan, et al., [159], Akele, et al., [160], Dawd, et al., [161] and Malbe, et al., [153]. However, the concentration of copper obtained in this study was in agreement with the average concentration of copper level reported by Intisar A. El Sharaa, et al., [156], and these values exceeded the maximum limit of copper in milk samples recommended by Standardization Administration of the People's Republic of China (0.01 mg/L) [162] and WHO/FAO (0.05 mg/L) [163], which might be attributed to environmental contamination [164, 165].

Manganese

The mean manganese concentration in the analyzed raw cow milk samples ranged from 0.052 to 0.0716 mg/L (Table 4.4). The highest mean manganese content were found in milk sample 3 and the lowest mean manganese content was found in milk sample 1. One-Way ANOVA analysis showed that there was a significant difference in the manganese content, $F(3, 32) = 6476.611$, $P < 0.001$, among the raw cow milk samples. However, Post hoc testing revealed that the mean calcium concentration obtained for milk sample 2 and milk sample 3 were statistically similar.

The results of the manganese content of the raw cow milk samples were much lower than the results reported by Hasan et al., as 0.112 mg/L for raw milk samples [159], and these values of manganese exceeded the maximum limit for manganese guideline values based on the World Health Organization [166]. 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.

4.6 Results of the Recovery experiment

Recovery experiment was carried out using selected raw cow's milk sample (Sample 1). The results of the %recovery experiment for the elements found in sample 1 were shown in Table 4.5.

Table 4.5: Recovery test results (mean ± SD, n=4, mg/L) for the analysed elements in sample 1

Metal	Conc. of unspiked sample (mg/L)	Amount added (50 µL of 1000 mg/L) = 10 mg/L	Conc. Spiked sample (mg/L)	Recovery (%)
Ca	1124.777± 2.770	10	1135.067± 2.240	102.880
Mg	103.550± 1.940	10	113.977± 1.710	104.220
Cu	0.082 ± 0.002	10	10.062 ± 0.013	99.810
Mn	0.052± 0.006	10	10.063± 0.004	100.110

The %recovery values for all the analysed elements lies within the range 99.810% to 104.220%, 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 RECOMMENDATIONS

This study provides information on the physicochemical parameters and elemental contents of raw cow's milk sold in informal markets in Hawassa city, Sidamma Region, Ethiopia. The result showed that the average values of physicochemical parameters such as pH, specific gravity, titratable acidity, total ash, and lactose were within the acceptable standard specified in Compulsory Ethiopian Drinking Water Specifications. However, the average values of total fat, total protein, and solid-not-fat were found to be below the recommended limits. Furthermore, the average water added content of the raw cow milk samples in the range of 5.54% to 10.33%. These might be due to the practice of adulteration of milk by water or other solid materials and other factors such as breed, feeding practices, or management practices, season and breed of the animals.

Metals such as Ca, Mg, Mn, and Cu were found and quantified in all raw cows' milk samples while the concentration of Ni, Co, Cr, and Pb were found to be below the detection limit of the instrument. However, further research should be conducted in order to make strong conclusion regarding the physicochemical quality and the metal content of the milk sold in informal markets in Hawassa city, Sidamma Region, Ethiopia.

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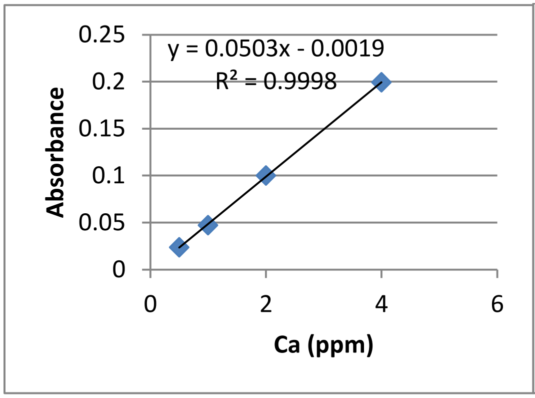
APPENDICES

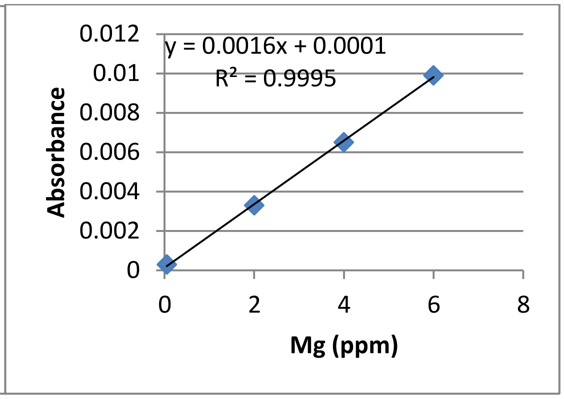
Appendex I: ANOVA analysis results for selected physicochemical parameters

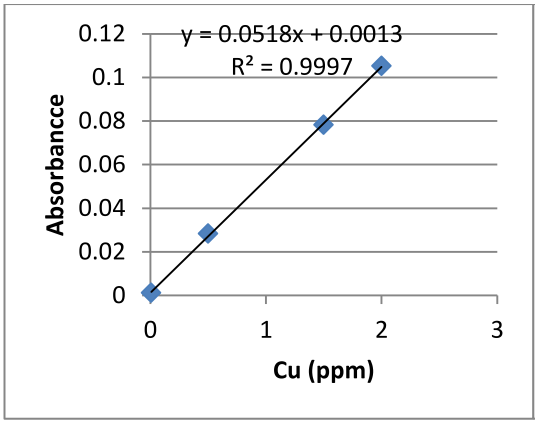
ANOVA

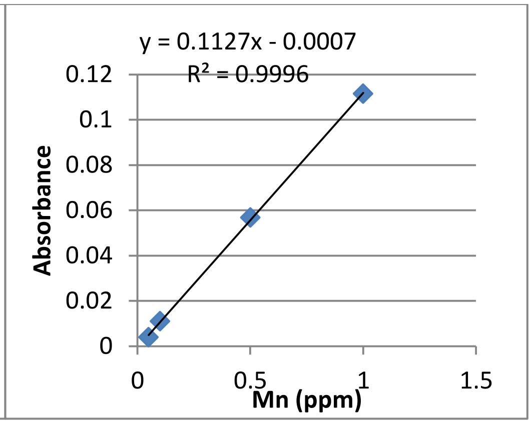
		Sum of Squares	df	Mean Square	F	Sig.
pH	Between Groups	.035	3	.012	36.377	.000
	Within Groups	.003	8	.000		
	Total	.037	11			
Water added in %	Between Groups	43.354	3	14.451	284.895	.000
	Within Groups	.406	8	.051		
	Total	43.760	11			
Density in g/mL	Between Groups	.000	3	.000	89.086	.000
	Within Groups	.000	8	.000		
	Total	.000	11			
Titratable acidity in % lactic acid	Between Groups	.006	3	.002	61.333	.000
	Within Groups	.000	8	.000		
	Total	.006	11			
Moisture in %	Between Groups	20.047	3	6.682	78.614	.000
	Within Groups	.680	8	.085		
	Total	20.727	11			
Total solids in %	Between Groups	20.047	3	6.682	78.614	.000
	Within Groups	.680	8	.085		
	Total	20.727	11			
Total ash in %	Between Groups	.002	3	.001	.913	.477
	Within Groups	.005	8	.001		
	Total	.007	11			
Solid-not-fat in %	Between Groups	.263	3	.088	.913	.477
	Within Groups	.767	8	.096		
	Total	1.029	11			
Total fat in %	Between Groups	17.429	3	5.810	331.984	.000
	Within Groups	.140	8	.018		
	Total	17.569	11			
Total protein in %	Between Groups	.035	3	.012	.913	.477
	Within Groups	.103	8	.013		
	Total	.139	11			
Lactose in %	Between Groups	.079	3	.026	.913	.477
	Within Groups	.232	8	.029		
	Total	.311	11			

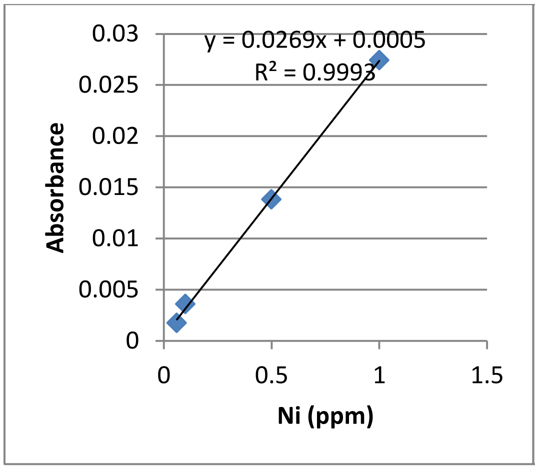
Appendix II: Calibration graphs

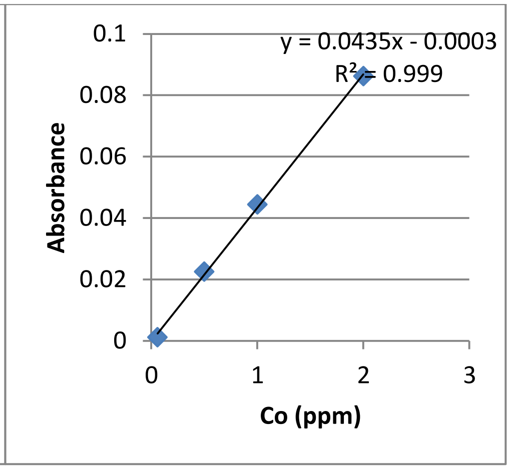


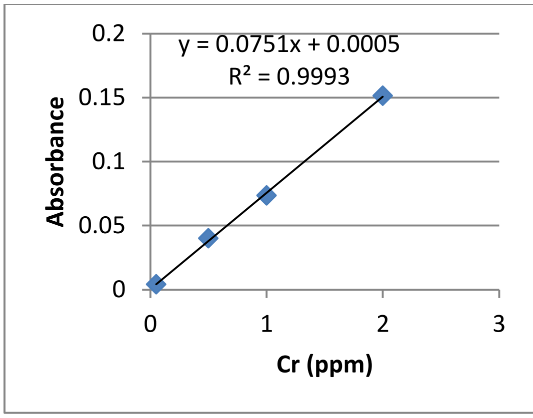


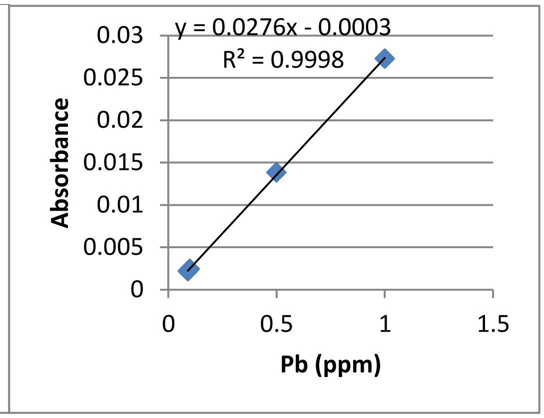









y = 0.0751x + 0.0005
R² = 0.9993
Absorbance
Cr (ppm)


y = 0.0276x - 0.0003
R² = 0.9998
Absorbance
Pb (ppm)

Appendix III: Optimization for the digestion of 5.0 mL of raw cow's milk samples

A) Optimization trials for different reagents ratios by making temperature constant

Trials	Volume of reagents (mL) $HNO_3:H_2O_2$	Digestion Temperature (°C)	Digestion time (hrs)	Observations
1	10:0	220	1:00	Deep yellow solution
			1:30	Deep yellow solution
			2:00	Light yellow solution
			2:30	**Clear solution**
2	8:2	220	1:00	Deep yellow solution
			1:30	Light yellow solution
			2:00	**Clear solution**
3	5:5	220	1:00	Deep yellow solution
			1:30	Deep yellow solution
			2:00	Light yellow solution
			2:30	**Clear solution**
4	5:1	220	1:00	Light yellow solution
			1:30	**Clear solution**
			2:00	**Clear solution**
5	4:2	220	1:00	Light yellow solution
			1:30	**Clear solution**
			2:00	**Clear solution**

B) Optimization of temperature

Trials	Volume of reagents (mL) $HNO_3:H_2O_2$	Digestion Temperature (°C)	Digestion time (hrs)	Observations
1	4:2	140	1:30	Light yellow solution
2	4:2	180	1:30	**Clear solution**
3	4:2	220	1:30	**Clear solution**
4	4:2	260	1:30	**Clear solution**

C) Optimization of digestion time

Trials	Volume of reagents (mL) $HNO_3:H_2O_2$	Digestion Temperature (°C)	Digestion time (hrs)	Observations
1	4:2	180	0:30	Deep yellow solution
2	4:2	180	1:00	Light yellow solution
3	4:2	180	1:30	**Clear solution**
4	4:2	180	2:00	**Clear solution**

D) Optimum conditions obtained through trials for raw cow's milk sample digestion

Sample volume (mL)	Volume of reagents (mL) $HNO_3:H_2O_2$	Digestion Temperature (°C)	Digestion time (hrs)	Observations
5.0	4:2	180	1:30	**Clear solution**

Appendix IV: ANOVA analysis results for selected metals

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Calcium (mg/L)	Between Groups	929580.528	3	309860.176	77196.999	.000
	Within Groups	128.444	32	4.014		
	Total	929708.972	35			
Magnesium (mg/L))	Between Groups	303.417	3	101.139	30.469	.000
	Within Groups	106.222	32	3.319		
	Total	409.639	35			
Manganese (mg/L)	Between Groups	.002	3	.001	6476.611	.000
	Within Groups	.000	32	.000		
	Total	.002	35			
Copper (mg/L)	Between Groups	.005	3	.002	570.108	.000
	Within Groups	.000	32	.000		
	Total	.005	35			