ADDIS ABABA UNIVERSITY

DEPARTMENT OF CHEMISTRY

Determination of the levels of selected trace heavy metals and fat content in commercially available milk brands in Addis Ababa city, Ethiopia.

By

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April, 2018

Determination of the levels of selected trace heavy metals and fat content in commercially available milk brands in Addis Ababa city, Ethiopia.

A Thesis submitted to the school of graduate programs of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry

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The determination of the levels of selected trace heavy metals and fat content in commercially available milk brands in Addis Ababa city, Ethiopia is my own work and that all sources of materials used for this work have been fully acknowledged. This work has been submitted in partial fulfillment of the requirement for Masters of Science in Chemistry degree at Addis Ababa University. I declare that this work is not submitted to any other institution anywhere for the award of any degree, diploma or certificate.

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LISTS OF ABBREVIATIONS AND ACRONYMS

AES -Atomic Emission Spectrometer

EFSA - European Food Safety Authority

ANOVA -Analysis of Variance

BDL - Below Detection Limit

EU - European Union

DEE - Di Ethyl Ether

DNA /RNA - Deoxyribo Nucleic Acid /Ribo Nucleic Acid

FAAS -Flame Atomic Absorption Spectrophotometer

HCL - Hallow Cathode Lamp

HTST - High Temperature Short Time

Ni-Cad - Nickel- cadmium

$PbEt_4$ - Tetra Ethyl Lead

PE - Petroleum Ether

% RSD - percentage Relative Standard Deviation

SNF - Solids Not Fat

UHT - Ultra High Temperature

WHO - World Health Organization

Determination of the levels of selected trace heavy metals and fat content in commercially available milk brands in Addis Ababa city, Ethiopia.

ABSTRACT

Six different pasteurized milk brands were analyzed for their trace heavy metals concentration and fat content. The concentration of heavy metals such as Zn, Cu, Cr, Cd, Pb and Ni was determined by using flame atomic absorption spectrophotometer. The elements Cu, Cr, Cd, Pb and Ni were not detected in all milk brands under the study. The concentration of zinc in Etete, Family, Harme, Shola, Holland and Mama milk brands were found to be 3.31± 0.671, 3.31± 0.408, 2.12 ± 0.692, 2.26 ± 0.224, 2.42 ± 0.731 and 1.93 ± 0.382 in μg/g, respectively. There is no significant difference at 95% confidence interval ($p > 0.05$) in the mean concentrations of zinc among the milk brands. The results obtained for the detected element in the present study were also compared with European Food Safety Authority maximum permissible limit to check whether it is below or above the set limit and were found below the allowed recommended limit. The validity of the digestion process was checked by recovery test. The percentage recovery of zinc was found to be 98.87% which is found in the acceptable range. The fat content of each milk brand was determined and found to be 2.4%, 2.8%, 2.8%, 2.8%, 2.6% and 3% in Etete, Family, Shola, Harme, Holland and Mama, respectively. The fat content of the different milk brands were found to be in the order of Mama > Family = Shola = Harme >Holland > Etete. The analysis of variance result showed that no significant difference was observed in the mean amount of fat content among all milk brands.

Key words: Heavy metals, Pasteurized milk, Acid digestion, FAAS, Fat content

1. INTRODUCTION

1.1. Background of the study

Milk is a complex, bioactive and white liquid substance that enhances growth and development of mammalian infants which can be considered as nearly nature's complete food as it is an appropriate source of proteins, fats, sugars, vitamins and minerals [1, 2, 3]. It is a characteristic secretion of normally functioning mammary glands of all the mammals which supplies the nutritional needs of the body better than any other single food stuff and can be considered as a meal on its own [4].

Milk and its products are the main constituents of the daily diets particularly for vulnerable groups such as infants, school age children, and elderly people [5]. It is the largest and the most important product with in the livestock sector and is the best and cheapest source of nutrition, easily accepted and used by all the age groups all over the world [6].

It is an excellent source of calcium, vitamin D, riboflavin and phosphorous and a good source of potassium, vitamin A, vitamin B-12 and niacin [7, 8]. It also provides moderate amount of magnesium, smaller amount of zinc and very smaller amounts of iron and copper to the body [9, 10].

According to its fat content, milk can be classified as whole milk, skimmed milk, semi- skimmed milk, low fat milk and standardized milk. Cow milk or whole or fresh refers to raw milk containing all of its constituents while skimmed milk refers to milk from which most of the fat has been removed and standardized milk refers to milk in which the fat content is adjusted to the predetermined value without changing any other constituents [11].

Human milk (breast feeding) is considered to be the best source of nutrition for infants. Milk based formulas which are the best substituent for breast milk are generally recommended when there is difficulty to bring up an infant on mother milk. Infant formulas are derived mostly from animals or plants and are mostly milk based or soy based formulation [12]. Milk has different physical properties like density, viscosity, color, electrical conductivity, freezing point, pH, etc, among these properties, the white color of the milk is due to so many fat globules (small round

particles) and colloidal protein molecules in milk scatters all the wave lengths of the white light causing the white color of the milk [13].

Although milk is an ideal source of micro and macro elements, additional amounts of contaminants like heavy metals might enter the milk and dairy products reaching levels that are harmful to humans. They can be contaminated either through water, food, manufacturing and packing process or the packing materials [1].

As an excretion of the mammary gland, milk can carry various xenobiotic substances (pesticides, antibiotics, drugs and heavy metals) and many environmental contaminants which are the risk factor for health and safety of the consumer [14]. The presence of heavy metals in dairy products may be attributed to the contamination of the original cow's milk which may be due to exposure of lactating cow to the environmental pollutants [15].

In chemistry heavy metal is a general collective term which applies to those metals which have density greater than 5 g/cm^3, their atomic weight ranges from 63.546 to 200.590 and their specific gravity greater than four; among heavy metals, copper, cadmium, chromium, lead, nickel and zinc are mentionable [8, 16]. Metals present in milk powder much below 50 mg/kg can be referred as trace metals [17].

Toxic heavy metals cause damages by enhancing the production of free radicals in several organs (brain, liver, kidney and heart) and interfering with cellular mechanisms against oxidation [18]. Their toxicity is largely related to age, sex, routes of exposure, daily intakes, duration of exposure and frequency of intake [15, 19].

At high dose even essential metals that are found in milk can cause toxicity to living organisms [16, 20]. As a result of environmental pollution by heavy metals that expose man and grazing animals to health risk, it becomes necessary to determine and control the level of these metals in milk and milk products because they significantly influence the human health particularly the developing infants and children [10, 21].

The determination of heavy metals can be performed by several instrumental techniques including photometric chromatography, ion chromatography, FAAS, inductively coupled plasma optical emission spectrometry, potentiometric stripping, capillary zone electrophoresis,

differential pulse anodic stripping voltammetry, mid IR spectrometry, particle induced X-ray emission and complexometric titration [5]. In the present study, FAAS is used to determine selected trace heavy metals in different milk samples.

1.2. Objectives of the study

1.2.1. General Objectives

The general objective of this project is to determine the levels of selected trace heavy metals present and fat content in commercially available different milk brands in Addis Ababa city, Ethiopia where milk is the main constituents of the daily diet for vulnerable groups such as infants, school age children and old age people.

1.2.2. Specific Objectives

The specific objectives of the study are:

i. To devise working procedure for the digestion of the milk samples.
ii. To determine trace heavy metals like Cd, Pb, Zn, Cr, Ni, and Cu in commercially available milk brands using flame atomic absorption spectrometer.
iii. To compare the amount of each heavy metals among the milk brands.
iv. To compare results of this research with internationally set limits (EFSA standard).
v. To determine fat content of the different milk brands.

1.3. Scope and Significance of the study

The levels of trace heavy metals in whole cows' and pasteurized milk were reported from different countries. The amount of cadmium, lead, zinc, chromium, copper and nickel were determined using different techniques [22, 23].

The levels of copper and zinc in cow's milk in Tanzania were below the toxic limit [5, 7]; the determined lead, cadmium and zinc in Turkey were much below the maximum level recommended [24]. But the concentration of lead in the dairy products in Iran was found to be greater than the EU value and the national Iranian standards reported in article [14]. Furthermore, in Ethiopia, the presence of these trace heavy metals were determined in bottled mango juices [25], in red peppers [26] and in honey [27]. However, there were no studies carried out in determining the levels of trace metals in commercially available pasteurized milk brands in

Addis Ababa city, Ethiopia. Therefore, this study focused in determining some of the essential and non essential trace heavy metals present as well as the fat contents in different milk brands. The study will have a significant contribution in understanding the levels of cadmium, lead, nickel, chromium, zinc and copper present in the different pasteurized milk brands so that the consumers will have information while using these products. They also will identify which type of milk brand is suitable for health in terms of metallic and fat content. The study also will pave the way for other researchers for further investigations.

2. LITERATURE REVIEW

2.1. Nutritional benefits of milk

Milk for bone health

The main dietary factors that affect the bone mass are calcium and vitamin D, although potassium, zinc, vitamin A and K and protein play a role. From milk, calcium, phosphorus and magnesium are the most important minerals to bone health, of which calcium is the most abundant [11].

Milk as a source of macro and micro nutrients

Milk intake may be a marker for diet quality due to its high nutrient content. It contains macro and micro nutrients that are very important for the normal functioning of the body [11].

Role of milk in treatment of under nutrition

Milk plays a very key role in treating under nutrition both in developed and developing countries. It has a positive effect on weight gain and linear growth in children aged six months to five years who are suffering from moderate malnutrition [11].

The possible mechanisms for cholesterol decreasing or removal by probiotic bacteria and fermented dairy products include inhabitation of intestinal cholesterol absorptions. The dairy proteins play an important role in food intake regulation and metabolic distracts relating to obesity. Whey protein in high protein milk products may improve insulin sensitivity and reduce fat deposition. Milk facilitates the maturation of digestive tube cell growth of a baby in gastro intestinal tract. In addition to its nutritional benefits, milk plays a significant role in controlling of chronic diseases, for example blood pressure can be treated with dairy products [28].

2.2. Chemical composition of milk

Milk is a complex material composed of several components that can be essential or non essential even though present in low concentrations [3]. On average milk is composed of 87% water, 4% to 5% lactose, 3% protein, 3% to 4% fat, 0.8% minerals and 0.1% vitamins per hundred grams of a milk sample [29 - 31].

2.2.1. Water

Water is the major component of all types of milks ranging from an average of 68% in reindeer milk to 91% in donkey milk [11]. This amount of water is controlled by the amount of lactose synthesized by the secretery cells of the mammary gland [30].

2.2.2. Lactose

Lactose is the major carbohydrate of milk. It is a disaccharide composed of D- glucose and D-galactose joined by a β-1, 4 glycosidic linkage. Lactose is only synthesized in the golgi vesicles of the lactating cells which provides a ready source of energy for the neonate [32].

2.2.3. Fat

Milk fat is often called “butter fat” is commercially the most valuable constituent of milk and is a great importance from the standard point of food value of the milk [6].
Fat provides our bodies with energy. It is a carrier of fat soluble vitamins and is responsible for their absorption. The nature of the fat depends on the types of fatty acids that it contains. The two types of fatty acids are saturated fatty acid and unsaturated fatty acids which are described by how the molecules in the fatty acid are joined together. In general the unsaturated fatty acids help to decrease our blood cholesterol levels while saturated fatty acids cause an increase in our blood cholesterol levels. An exception to this rule is the trans fatty acids or bad fats which are unsaturated fatty acids that increase our blood cholesterol levels [33].

Milk fat is one of the most complex of all common fats, composed of many fatty acids mainly saturated (66%), mono unsaturated (30%) and polyunsaturated (4%). All short chain (4:0 to 10:0) and half of the medium chain (12:0 to 17:0) fatty acids in milk fat are synthesized from acetate and β-hydroxy butyrate in the mammary gland epithelial cells. The other half of medium chain and almost all long chain (18:0 and longer) fatty acids are derived from blood plasma fatty

acids of dietary origin or from mobilization of stored body fat. Milk fat contains substantial amount of short chain fatty acids, making it unique compared to other fats. These short chain fatty acids especially butyric acid (4:0) are important for flavor development in some cheese and fermented dairy products [34]. Milk fat contains approximately 400 different fatty acids which make it the most complex of all natural fats [11].

2.2.4. Protein

Proteins are among the most complex of organic substances that contain carbon, hydrogen, oxygen, nitrogen, sulphur and sometimes phosphorous [6]. Milk is generally considered as important source of protein in the human diet providing approximately 32 g of protein per liter. Its protein fraction can be soluble whey protein (20% of milk protein fraction) or insoluble caseins (represents 80%) [29].

2.2.5. Vitamins and Minerals

The milk vitamin profile includes liposoluble (A, D and E) and hydro soluble vitamins (B-complex and vitamin C). The concentration of fat soluble vitamins in milk depends on the content of milk fat and thus low fat and skim milk have lower amount of vitamin A, D and E. For this case it is possible to fortify the skim milk with vitamin A and D to improve its nutritional richness [29]. The mineral composition of cow's milk consists of calcium, iron, magnesium, phosphorous, potassium, sodium, zinc, copper, selenium and manganese, etc. Among these milk minerals, potassium, calcium and phosphorous are in larger amount [11].

The chemical composition of milk can be affected by many factors, like species, genetics (breed), feed, stage of lactation and season [29, 35].

Species

Milk can be obtained from different species such as goats, sheep, camels, cows, etc and the composition of the milk that is obtained from these different species varies as the species vary [30]. Table 1 shows the chemical composition of milk in different species.

Table 1. The composition of milk from different mammals in g/100 g of milk [30].

Species	Water	Proteins	Fat	Lactose	Ash
Cow	87.2	3.5	3.7	4.9	0.72
Sheep	82.7	5.5	6.4	4.7	0.92
Goat	86.5	3.6	4.0	5.1	0.82
Camel	87.7	3.5	3.4	4.7	0.71

Genetics

Animals of the same species having different breed have different milk composition [35]. Table 2 shows typical fat, protein and lactose contents (g/100 g) for the milk of several breeds of dairy cows.

Table 2. Fat and protein composition of different breeds of dairy cows [35].

Breed	Fat (g/100 g) of milk	Protein (g/100 g) of milk	Lactose (g/100 g) of milk
Tharparkar	4.37	3.92	5.35
Karan Fries	3.91	3.58	5.39
Sahiwal	4.23	3.6	5.38
Assam native cattle	5.34	3.04	-

Feeds

An increase in feed intake usually results in the production of greater volume of milk. As cows consume more energy than they use, body weight is regained, losses in body condition are minimized and cows produce milk of normal fat and protein content. Nutritional strategies that optimize rumen function also maximize milk production and milk components. In general, as energy intake or ration density increase and fiber decreases, milk fat content will be reduced, while protein content is increased. In contrast, as ration fiber levels increase and energy is reduced, milk protein is depressed and milk fat is increased. Fat concentration is most sensitive to dietary change [31].

Stage of lactation

In the early lactation, fat and protein decrease and lactose concentration increases, whereas in the late lactation, fat and protein increase and lactose decreases [35].

Season

The lactose content was less, in winter compared to summer where as the percentage of milk fat and protein are high in winter compared to summer. This variation is related to the changes in both the types of feed available and climatic conditions. Hot weather and high humidity decrease dry matter intake and increase feed sorting, resulting in lower forage and fiber intake [35].

There is a negative correlation between environmental temperature and the amount of milk fat and protein; when the temperature is increased the solid fat tends to decrease. Milk fat is the most variable component among the milk contents. The amount of milk fat can be affected largely by seasonal variation [36].

Livestock performance is affected by heat stress because an animal having difficulty in losing heat will decrease its heat production by lowering feed intake and this situation in turn results in lowering milk production. Most of the time the upper critical air temperature for a lactating cow is in the range of 24 ^{o}C to 27 ^{o}C [37].

2.3. Milk Adulteration

Adulteration is the act of adding substances to a product that makes it unfit for consumption. These impurities are added to substitute the contents of the product at a cheaper rate to increase the quantity. Milk adulteration is one of the most common and old form of adulteration. It is done not only intentionally but also incidentally through contamination during the process of preparation, storage and transportation. Adulterated milk has adverse effect on health because of the toxic nature of the substituting compounds or lack of compounds of nutritional value [38].
Milk is very easily adulterated throughout the world significantly worst in developing countries due to the absence of adequate monitoring and lack of proper law enforcement. The possible reasons for this may be demand and supply gap, perishable nature of milk and lack of suitable detection tests [39]. The typical adulterants include:

Water

Water is the most common adulterant added to increase the volume of milk which in turn decreases the nutritive value of milk. If impure water is added, it causes water born diseases [39, 40].

Urea

Urea is added to milk to provide whiteness and this increases non protein nitrogen content but addition of urea is harmful to heart, liver and especially for kidney as kidney has to do more to remove urea from the body [39, 40].

Hydrogen peroxide (H_2O_2)

It is added to milk to prolong its freshness, but its addition to milk damages the gastro intestinal cells which can lead to gastritis and inflammation of the intestine [39, 40].

Starch

Generally sugar is mixed with the milk to increase the SNF content of the milk, i.e, to increase the lactometer reading of milk which was already diluted with water [39, 40].

Chlorine

Chlorine is added to compensate the density of the diluted milk after addition of water but chlorinated milk can cause clogging in arteries and develop heart problem. It also disrupts the acid- base balance and the blood pH in the body [39, 40].

Antibiotics

These are added to treat cows' diseases and 80% of the veterinarians use them to treat mastitis disease. They can exist in the form antimicrobial residues in milk and cause allergic reaction and tissues damage [39, 40].

Preservatives

Sodium carbonate, sodium bicarbonate, boric acid, formalin, benzoic acid, salicylic acid and sodium azides are added to milk to preserve the milk for long time but their presence in milk has poisonous effect which can lead to death, diarrhea and vomiting [39, 40].

2.4. Milk structure

Milk is a dispersion of fat globules (fat particles) and casein micelles (protein particles) in a continuous phase of water, sugar (lactose), whey proteins and minerals. Milk minus fat globules is milk plasma. Casein micelles consist of water, protein and salts. The protein is casein which is present as caseinate. It binds cations primarily calcium and magnesium. Milk serum or the liquid in which the micelles are dispersed is milk minus fat globules and casein micelles [32]. Figure 1 describes magnification of milk to visualize its structure.

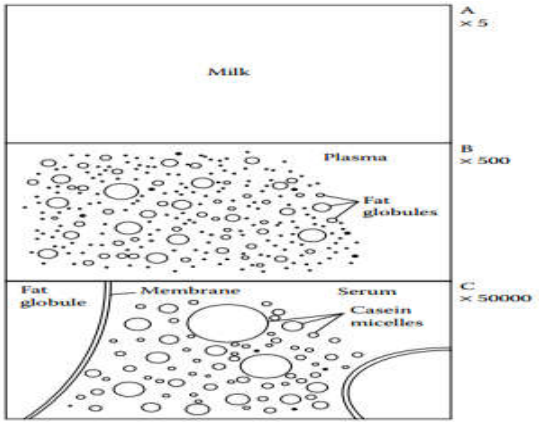

Figure 1a

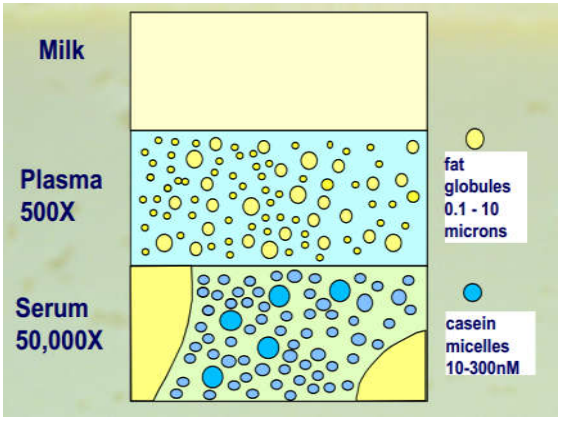

Figure 1b

Figure 1a and 1b. Milk viewed at different magnifications, showing the relative size of structural elements using electron microscope [32].

(A) Uniform liquid

(B) Spherical droplets consists of fat

(C) The plasma contains proteinaceous particles, which are casein micelles.

2.5. Milk production in Ethiopia

Ethiopia holds the largest live stock population in Africa [41, 42, 43]. The total cattle production for the country is estimated to be 52.13 million and the total annual national milk production comes from about 10 million milking cows and is estimated 3.2 billion liter [43].

Based on the location, milk production system can be urban, peri-urban and rural. Both urban and peri-urban systems are located near Addis Ababa city and take the advantage of urban markets. The urban milk system consists of 5,167 small, medium and large dairy farms producing about 35 million liters of milk annually. Of the total urban milk production, 73% is sold, 10% is for house hold consumption, 9.4% for calves, 7.6% processed for butter and cheese. The peri-urban milk system includes small holders and commercially dairy farmers in proximity of Addis Ababa and controls most of the country's improved diary stock where as rural diary system includes pastoralists which is not market oriented and is remained for home consumption [41].

Oromia region supplies more milk to the market compared to other regions where Addis Ababa is the dominant market compared to other towns of the country. Even if Ethiopia has the largest milk producing cattle, it has low level of milk consumption compared to Kenya (90 lt/cap) and Uganda (50 lt/cap) with national per capita consumption of milk and milk products is estimated at 17 kg [44].

2.6. Milk processing

The principal raw material required for the production of pasteurized milk, butter, cheese and yoghurt is raw milk. In the production process, small quantities of coagulation enzymes and salt are required. The raw (whole) cow milk and salt are available locally while the coagulation enzymes have to be imported. The auxiliary materials required for the envisaged plant comprise packing materials like 500 cc plastic bags, 40 gm glycine paper and carton box. The plastic bags and carton boxes can be acquired from the local market while the glycine paper has to be imported.

Processing of raw milk mainly involves heat treatment operation usually known as pasteurization and sterilization. A weighed amount of raw milk is pumped to a clarifier by means of the milk pump, where it is removed of microscopic impurities. Clarified milk is then sent to the cooler

where it is cooled to about 2-5 ^{o}C, then pumped to the storage tank. The milk is, then, preheated and pasteurized to a temperature of about 80 ^{o}C by heat exchange. Further, by the effect of ultra-high temperature sterilizer, the fatty ingredients are homogenized in the homogenizer and recycled to the ultra-high temperature sterilizer where it is pasteurized instantly in about 2 seconds at high temperature of 135 ^{o}C. Finally, cooling is achieved by means of chilled water to lower the temperature to 3 ^{o}C, after which the milk is stored in the surge tank for filling into suitable containers for various uses. After such a process, a specified quantity of the milk is sold as a pasteurized product while the remaining portion is further processed in the plant for the production of other milk products such as butter and cheese [45].

2.7. Milk preservation methods

Milk is almost sterile when secreted from a healthy cow udder. The natural inhibitors in milk such as lactoferrin and lactoperoxidase prevent significant rise of bacteria numbers for the first three to four hours after milking at ambient temperatures. Cooling to 4 ^{o}C with in this period maintains the original quality of the milk [46, 47].

Everybody knows very well how quickly the milk becomes sour when it is stored for a long time at high surrounding temperature. This is due to the inherent lactic acid bacteria contaminating microorganisms from storage vessels or environment break down the lactose in milk to lactic acid. When more lactic acid is accumulated, the milk become sour and coagulates. Whether milk is directly sold to consumers or processing in factory, it must be handled hygienically. Hygienic milk handling includes clean equipments, clean milking environment, good personal hygiene and preserving the milk during storage and transportation to consumers or processing plants [47].

The most common methods of milk preservations are

Cooling

Cooling prevents the growth of bacteria in milk to maintain its quality for domestic consumption or during transport to the processing plants. Cooling does not reduce bacteria numbers but slows down their growth [47].

Pasteurization

It is heating every particle of the milk or milk products to a specific temperature which destroys all the pathogenic microorganisms without seriously affecting its composition to 63 ^{o}C for 30 minutes [47, 48], or to 72 ^{o}C for 15 seconds [47]. Batch Pasteurization is suitable for small scale producers and farmer cooperatives that involve heating the milk for 63 ^{o}C for at least 30 minutes. High Temperature Short Time (HTST) is applicable for processing large quantities of milk (more than 250 liters at a time) with heating at 72 ^{o}C for 15 seconds and Ultra High Temperature (UHT) involves heating the milk for 135 ^{o}C for two seconds and is used by large factories with special machinery. In this process the milk can be stored for six months even without refrigeration [48].

A more moderate heat treatment like 65 ^{o}C for 15 seconds called thermalization which reduces the number of psychrotrophs leaving most milk enzymes and agglutins intact. It is a far better method for controlling the quality of dairy products than cooling the raw milk [32].

Sterilization

Sterilization of milk is aimed at killing all micro organisms, including bacterial spores, so that the packaged products can be stored for a long period at ambient temperature, without spoilage by microorganisms [32]. It is done at 121 ^{o}C for 5 min in an autoclave [49].

2.8. Sources of heavy metals

Heavy metals can be considered as deadly toxins that are accumulated (bioaccumulation) in plants and animals tissues through biological cycle. They are persistent contaminants in the environment; air, water and soil and are dangerous substances causing series health risks [50, 51]. When heavy metals enter to human body, they disrupt normal cellular processes leading to toxicity in a number of organs. Their accumulation in different tissues is due to once if they are taken up in to the body and stored in a particular organ like liver and kidney, they will be excreted at low rate compared with their uptake [52]. All the sources of heavy metals can be either natural or anthropogenic sources [7, 8]. An example of natural processes is geological deposition of metals by erosion in ground water, weathering of minerals, volcanic activities and forest fires are natural phenomena that release heavy metals to environment [8, 50]. Human

activities such as mining of minerals and ores, smelting of metals, exploration of energy resources like coal, oil and natural gas, waste disposal, waste incineration, urban effluents, traffic emissions, fertilizer applications and long term application of waste water in agricultural lands, modern products like cosmetics, mercury amalgamation dental filling represent anthropogenic contribution to the environment [53 , 54].

Heavy metals are environmental unfriendly pollutants that can cause adverse effects on living organisms. They can enter to the human body either through inhalation or ingestion [16, 53]. Due to an increase in agricultural and industrial activities the concentration of heavy metals in the environment increases. When these metals are taken in by plants through absorption, they will be transfer to the plants. Animals that graze on such contaminated plants receive the toxic heavy metals that can result contaminations on milk and milk products and hence the cycle of theses metals in the environment is linked with the food chain as soil-plant-animal-man [7]. Unlike organic pollutants which can be broken down, metals cannot be degraded or metabolize and will remain in the soil permanently [53].

Measurements of minor and trace heavy metals contents are very important to assess the quality of milk during its manufacturing, treatment and production [55]. These metals present in milk can be removed using nano sized metal oxides such as iron oxide, manganese oxide, aluminum oxide, titanium oxide and cerium oxide which have particularly high level with strong tendency to absorb heavy metals however titanium dioxide is mostly used as a suitable material to remove these pollutants [51].

2.9. Essential and non essential metals

Based on the nutritional value, metals from food products can be categorize as essential (Na, K, Ca, Cu, Zn, Cr, Ni and Mn) and non essential metals (Pb,Cd, Hg, Ag, Sn, Al). The essential metals are important to maintain proper metabolic activity in living organisms where as the non essential metals have no biological role and can cause impairment. For both classifications the increase in the concentration of the metal in food over the limits can cause toxic effects for the consumers of these products [5, 7, 56].

2.10. Roles played by essential metals

2.10.1. Nickel

Little amount is needed by the human body to create red platelets [50]. Natural sources of nickel includes dusts from volcanic emission and weathering of rocks and soils, but in water it is derived from biological cycles and solubilization of nickel compounds from soil and from sedimentation of nickel from the atmosphere . Nickel compounds are used for electroplating, Ni-Cad alkaline batteries and catalysts. The most hazardous routes of nickel exposure are by inhalation, ingestion or skin contact in nickel and its alloy production plants. Inhalation of nickel causes irritation of nose and sinus and leads to loss of sense of smell [57]. The most common adverse health effect of nickel in humans is an allergic skin reaction [58, 59]. Its toxicity leads to cancer and long term exposure damages the heart, lungs and nasal cavity and adversely harms the kidney, blood, liver and immune system [59].

2.10.2. Zinc

Zinc is essential for growth and development. It is a constituent of more than 200 metallo enzymes, many of which regulate carbohydrate, lipid and proteins. It plays vital roles in the synthesis of genetic material and regulation of gene expression and cell division, epithelial integrity, cellular immunity and sexual maturity. It is also used for the synthesis, storage and secretion of insulin. Infants need high zinc to support their rapid growth. Iron supplementation could decrease zinc absorption [60]. The adult population mean intake of zinc should not exceed 45 mg/day in order to avoid zinc- related interactions. Its shortage may result in delayed growth and slow maturation [61].

2.10.3. Copper

Copper is a component of essential enzymes (cuproenzyme) and is involved in respiration and synthesis of hemoglobin [55]. It is an important metallic activator of several enzymes. Diets which contain excess copper have been reported to lead to toxicity and liver damage during childhood [60].

The main anthropogenic sources of copper are pesticides, fertilizers, ore mining and smelting [50].

2.10.4. Chromium

Human activities that contribute chromium to the physical environments are tanneries, steel and industries fly ash. Long haul presentation can bring about kidney and liver harm, and harm excessively circulatory and nerve tissue [50]. Although Cr^{3+} has been proven to be essential for biological path ways like glucose metabolism, Cr^{6+} is the most toxic and carcinogen, allergen and acute irritation in humans. Its greater toxicity is due to its reduction to +3, +4 and +5 intermediates that induce free radical which can bind to intracellular macromolecules [62].

2.11. Toxicity and harmfulness of non essential metals

2.11.1. Lead

Lead is an ubiquitous heavy metal released in small amounts in to environments by natural processes. Young children undergo rapid development and consequently are more susceptible than adults to the effect of lead. It impairs central nervous system, especially in infants and young children during the critical stages of brain development. Lead exposure in children at minimal levels can be associated with intellectual and cognitive deficits characterized by reduction in intelligence quotient (IQ), short attention span, reduced short term memory, reading and learning disabilities [59]. Human exposure to lead comes from the main sources such as leaded gasoline ($PbEt_4$), lead based paints, having lead pipes in water supply system and exposure to lead mining, smelting and coal combustion [56].

The main uses of lead are industrial like lead storage batteries, alkyl lead production as an additive to petrol used as anti knocking agent [53].

2.11.2. Cadmium

Much of cadmium entrance to atmosphere is from incineration of ferrous scraps and metallurgy processes. It is primarily comes from electroplating, plastic manufacturing, mining, paint pigment, alloy preparation and Ni-Cad batteries [19]. It can replace some bone enzymes and causes deformed bones, short stature, bone fracture, birth defects, decrease in fetal weight, abnormalities in the DNA and fetal proteins and its high infection leads to abortion [51]. Rice is able to transfer high level of cadmium from soil in a bio available form. Livestock provide an important break in the food chain from soil to humans because liver and kidney are effective

filters and little of cadmium reaches to meat and milk [53]. As the kidney accumulates cadmium during life, it is the main target organ in chronic environmental exposure [51, 53].

3. EXPERIMENTAL

3.1. Equipments and Reagents

3.1.1. Equipments

A refrigerator, digital analytical balance with a precision of ± 0.0001 g, 2 mL, 5 mL and 10 mL pipettes, 1-10 µL, 5 µL, 10 µL, 20-200 µL and 1000-5000 µL micro pipettes, measuring cylinders, 25 and 100 mL volumetric flasks, 250 mL round bottom flasks (24/29) fitted with reflux condenser, Kjeldahl digestion block and Atomic Absorption Spectrophotometer (ZEE nit 700P) with air-acetylene flame at selected wave lengths.

3.1.2. Chemicals and Reagents for metal determination

Reagents that were used for analysis of the selected metals were all analytical grade. 69-72% HNO_3 and 70% $HClO_4$ were used for the digestion of milk samples, distilled water was used to wash apparatuses and deionized water was used throughout the study to rinse apparatuses and for the dilution of the digested samples and standard solutions. All glasswares and plastic containers were washed with detergents. The standard stock solutions containing 1000 mg/L in 2% HNO_3 were taken to prepare intermediate standard solution of concentration 10 mg/L, where working calibration standard solutions were prepared from it for the determination of metals in the spiked and non spiked samples.

3.1.3. Chemicals and Reagents for fat content determination

Reagents that were used for the determination of fat content in six different milk brands were HCl (1.18 sp. gr) used to dissolve and separate the milk protein from fat, ethyl alcohol (absolute anhydrous) which dehydrates the protein by removing the bonded water and adjust the polarity of the aqueous phase to promote a clear interface between the organic and aqueous phases, diethyl ether (peroxide free) which is an efficient fat solvent, but it has some affinity to water and ethanol, petroleum ether (boiling point 40-60 ^{0}C) is a non polar fat solvent and water repellent. It renders ethereal phase free of water.

3.2. Procedures

3.2.1. Apparatus Cleaning

3.2.1.1. Apparatus cleaning for metal determination

The cleaning of any apparatus that is used in this practical activity is the primary task to avoid contamination as the project is focusing on analysis of trace metals in trace level. Hence, the apparatus such as volumetric flasks, pipettes, measuring cylinders, round bottom flasks (24/29), beakers, funnels, plastic bottles and scissor were first washed with detergent and tap water rinsed with distilled water followed by deionized water and soaked in 20% diluted nitric acid (30% for plastic bottles) for 24 hours, rinsed with deionized water and allowed to dry at room temperature for use.

3.2.1.2. Apparatus cleaning for fat content determination

Apparatus such as beakers, measuring cylinders, pipettes, syringe, graduated plastic tube and conical flasks were first washed with detergents and tap water, then rinsed with distilled water followed by deionized water and allowed to dry. The washed apparatuses were also rinsed with 100 mL of 1:1 ratio of petroleum ether and diethyl ether to remove any fat adsorbed on their internal surface. After some time the ethers were evaporated easily at room temperature and eventually the apparatuses were ready for use.

3.2.2. Sample Collection

For this study, six different commercially available pasteurized milk brands packed in Addis Ababa city, Ethiopia were purchased from super markets located at different areas of the city. All of them were packed in plastic bags. Since the milk brands have short shelf life, one brand of milk samples were purchased and stored in refrigerator at a time until analysis. Three plastic bags of the same brand for each of the six different milk brands (a total of 18 plastic bags) were used for the determination of heavy metals and fat content. For fat content determination, the purchased samples were used immediately as the fat is sensitive for reaction.

3.2.3. Sample Preparation

3.2.3.1. Sample preparation for metal determination

For each of the different pasteurized milk brands, three plastic bags (500 mL each) of the same brand were used. All the three plastic bags of the same brand were teared with acid washed scissors and 100 mL of sample from each bag was taken using a measuring cylinder so that a total of 300 mL of milk from the same brand was transferred to a plastic bottle for homogenization to get representative sample. After the milk is mixed thoroughly, 1 g of milk was measured and transferred to a round bottom flask (24/29) for digestion. In figure 2, each plastic bottle contains a mixture of the three plastic bags of the same milk brand purchased from different supermarkets.

Figure 2. Preparation of homogenized milk samples for digestion.

3.2.3.2. Sample preparation for fat content determination

In fat content determination, 100 mL of milk sample was taken from the plastic bags using measuring cylinder and transferred to a plastic bottle. After mixing thoroughly, the samples were made ready for extraction.

3.2.4. Working procedure for metal determination

It is important to develop an optimum working procedure to obtain reliable result. Accordingly, article [63] was modified to prepare a clear and color less digested milk sample solutions and this modified digestion procedure was employed using 69-72% HNO_3 and 70% $HClO_4$ mixtures by varying the volume, percentage and the time required to obtain clear and color less solution by keeping the temperature dial constant. Up on investigating the nature of the final digests obtained, the modified procedure was finally applied.

3.2.5. Digestion of milk samples for metal determination

Exactly 1.000g of milk samples were taken from each of the homogenized plastic bottles and transferred to a digestion round bottom flask to which 2.5 mL of 69-72% concentrated HNO_3 and 1 ml of 70% $HClO_4$ were added to the sample. The sample was swirled gently to homogenize the mixture and then fitted to a reflux condenser. Then, the digestion flask containing the sample and the reagent mixture was digested in a Kjeldahl digestion block fitted with reflux condensers for two hours at a temperature dial of 8 (240 ^{o}C). After cooling the content for 15 minutes at room temperature without removing the condensers, 1 mL of 69-72% concentrated HNO_3 and 1 mL of 70% $HClO_4$ were added and the content was redigested for an additional one hour at the same temperature dial and eventually clear and color less solution was obtained. After cooling the digested clear solution for 15 minutes, the flasks were detached from reflux condenser and 5 mL of deionized water was added in to it and transferred to a 25 mL volumetric flask using filter paper (Watman No. 41). The round bottom flasks were also again rinsed with 5 mL of deionized water to take the remaining metal ions and made up to the mark with deionized water. Each milk brand was digested in triplicates and hence a total of eighteen digests were made ready from the six milk brands for analysis of their metal contents.

Digestion of blank solutions was also done by keeping all the digestion parameters constant. Thus, for the analysis of milk samples six blank solutions were prepared. All the digested samples were then stored in a refrigerator until analysis using FAAS.

3.2.6. Acid digestion method for fat content determination

For the determination of the fat content of each milk brand, the procedure in the reference [64] was used. Accurately weighed 5 g of the homogenized milk sample from each brand was taken from the plastic bottle and transferred to a 100 mL small beaker. 5 mL of concentrated HCl (sp. gr = 1.18) was mixed with the milk sample and was heated on a Bunsen burner by stirring continuously with glass rod until dark brown solution appeared. After cooling at room temperature, the contents were transferred to a graduated plastic tube (Figure 3). Addition of 5 mL ethyl alcohol and 13 mL of PE and DEE each in to a plastic tube followed by vigorous shaking for one minute gave two separated layers shown in Figure 3. The colorless upper layer of the mixture which is a mixture of ethers and fat was decanted in to a conical flask and the solvents were evaporated in water bath at a temperature that does not cause bumping. The fat was dried in an oven at 102 ^{o}C until constant mass was obtained. The oven drying removes the last traces of water, alcohol, solvents (PE and DEE) and finally the percentage of fat was determined. Figure 3 shows the extraction of fat from pasteurized milk samples.

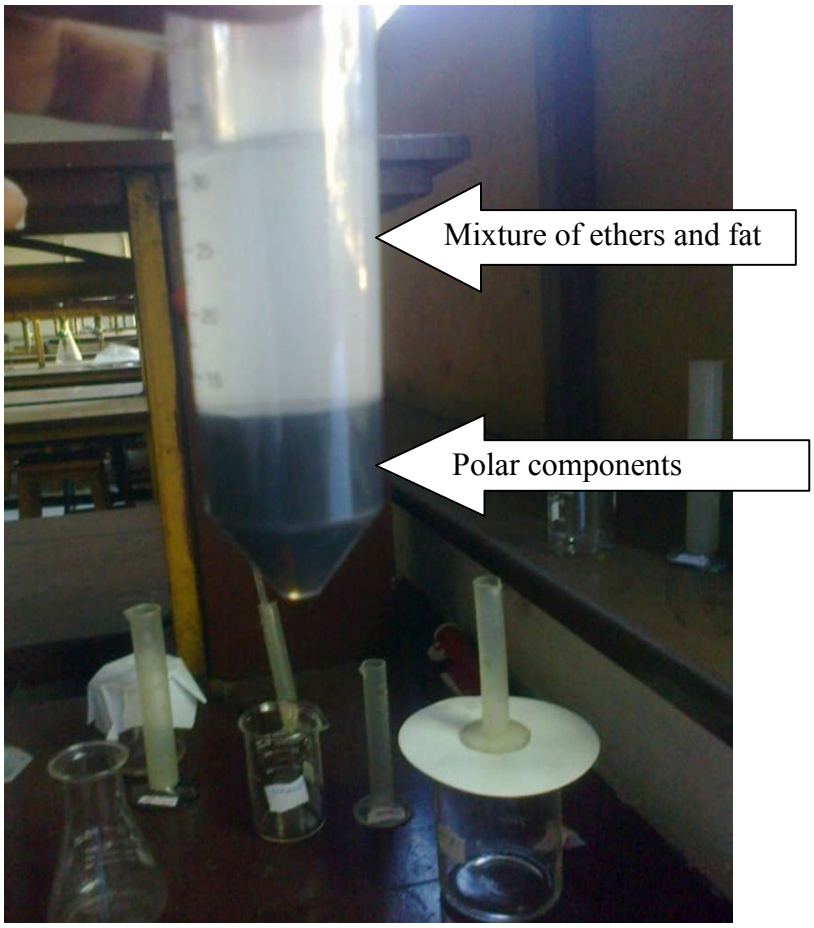

Figure 3. Acid digestion extraction of fat from pasteurized milk samples.

3.2.7. Determination of the metals

Intermediate standard solutions having 10 mg/L of metal of interest were prepared from atomic absorption spectroscopy standard stock solutions that contained 1000 mg/L. The already obtained 10 mg/L secondary standard solutions were further diluted with deionized water to obtain four working standard solutions of Zn, Pb, Cu, Ni, Cd and Cr and were analyzed with FAAS equipped with air- acetylene flame system after the instrumental operating conditions were optimized for maximum signal intensity of the instrument. Hallow cathode lamp (HCL) for each metal (Zn, Pb, Cu, Ni, Cd and Cr) operated at the manufacturer's recommended conditions were used at its corresponding primary source lines. The same analytical procedure was applied for the determination of metals in the digested blank solutions.

3.2.8. Digestion of milk samples spiked with standard metal solutions

Due to the absence of certified reference materials for the pasteurized milk brands in our laboratory, the validity of the modified procedure for milk samples was checked by performing spiking. Known amount of zinc from 100 mg/L intermediate solution made from 1000 mg/L by dilution was added to flasks containing one gram of milk samples. For zinc, 40 % of its AAS result was added to the triplicates by measuring 13.24 µL from 100 mg/L intermediate solution. Both the spiked and unspiked triplicate samples were digested simultaneously based on the modified developed procedure. The digests were transferred to 25 mL volumetric flask and diluted to its mark with deionized water. Finally, the solutions were analyzed for metal concentration with FAAS and the percentage of recovery was calculated. Table 3 shows the amount of standard solutions required for spiking.

Table 3. Amount of intermediate standard solutions added to spike the Etete milk samples.

Metal	Average conc. of AAS (mg/L)	40% conc (mg/L)	Intermediate standard soln.(mg/L)	Volume of intermediate standard soln. added (µL)
Zn	0.13	0.05	100	13.24

3.2.9. Method detection limit

Method detection limit is the smallest mass of analyte that can be distinguished from statistical fluctuations in the blanks, which usually corresponds to the standard deviation of the blank solution times a constant [25] or it is the amount of analyte that gives a signal equal to three times the standard deviation of the blank [25, 27]. Instrument detection limits were directly obtained from the instrument manual for each element under study. In this study, after the digestion of six blank solutions containing HNO_3 (69-72%) and $HClO_4$ (70%), six readings were taken from AAS and the standard deviation was calculated.

The method detection limit of the metal was obtained by using the equation used in reference [25]:

$$MDL = 3 \times \sigma_{blank}$$

Where σ is the standard deviation of the blank reading

Based on the above equation, the method detection limit of Zn metal was found to be 0.12 mg/L.

4. RESULTS AND DISCUSSION

4.1. Calibration of the instrument

Calibration curves were drawn to determine the concentration of trace metals in milk sample solutions. To do this, series of working standard solutions were prepared from 10 mg/L intermediate standard solutions of the respective metals. After the instrument is calibrated properly, the concentration of metals in each sample was measured. The correlation coefficient (R^2) of the calibration curves of each metal was determined by plotting working standard concentration (mg/L) versus their corresponding absorbance. The working standard solutions and the correlation coefficient (R^2) obtained from the calibration curves of the analyzed metals are summarized in table 4.

Table 4. Concentrations of working standard solutions, intermediate solutions and the correlation coefficients of the calibration curves for the respective metals.

Metal analyzed	Concentration of working standard solutions (mg/L)	Concentration of intermediate standard solutions (mg/L)	Correlation coefficient (R^2)	Regression equation
Zn	0, 0.15, 0.5, 0.75, 1	10	0.995	Y= 0.086x+0.000
Cu	0, 0.25, 0.5, 1, 2	10	0.996	Y= 0.022x-0.000
Cr	0, 0.5, 1, 1.5, 2	10	0.998	Y= 0.012x-3E-05
Cd	0, 0.25, 0.5, 0.75, 1	10	0.998	Y= 0.044x-0.001
Ni	0, 0.5, 1.5, 3, 6	10	0.999	Y= 0.006x-0.000
Pb	0, 0.4, 0.8, 1.2, 1.6	10	0.998	Y= 0.003x+6E-05

The calibration graph of each analyzed metal is shown in figure 4.

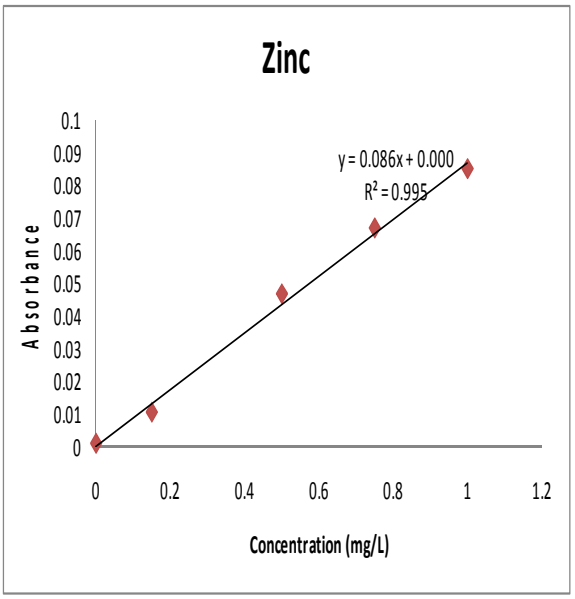

Figure 4a. Calibration curve for Zn

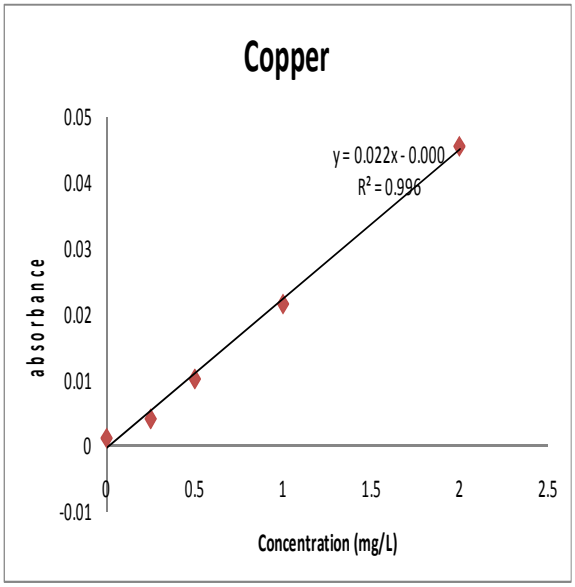

Figure 4b. Calibration curve for Cu

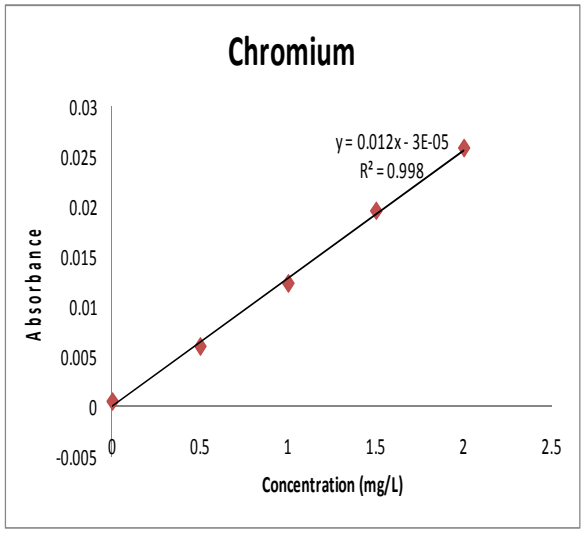

Figure 4c, Calibration curve for Cr

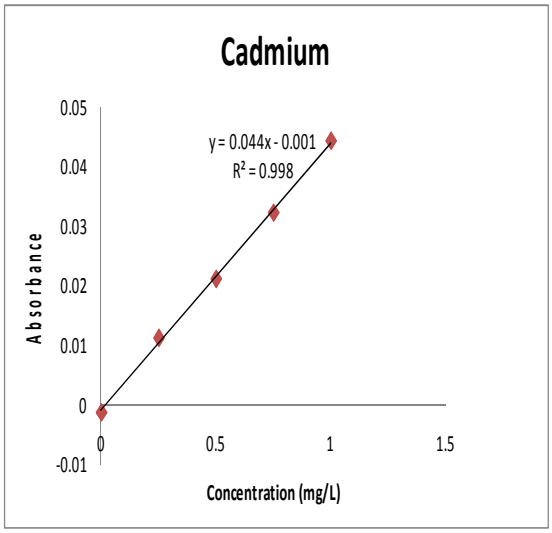

Figure 4d. Calibration curve for Cd

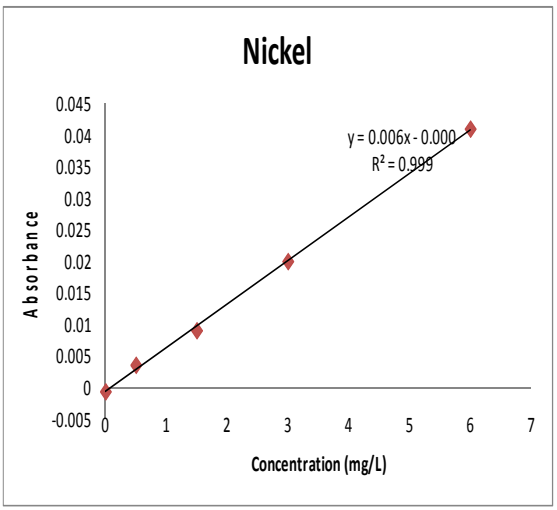

Figure4e. Calibration curve for Ni

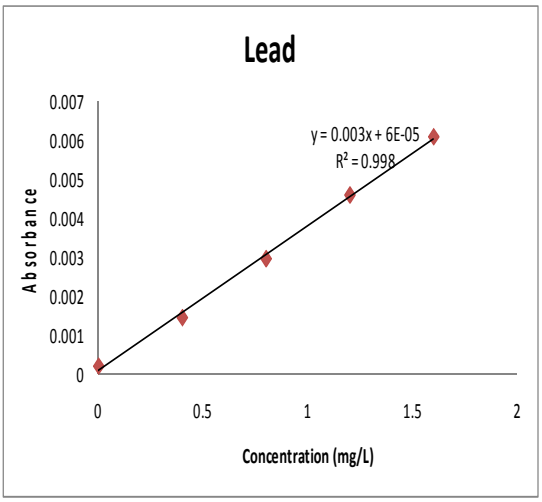

Figure 4f. Calibration curve for Pb

4.2. Digestion procedure for metal determination

For milk samples, the digestion condition was adjusted by modifying the procedure used in the reference [63]. The modified procedure mainly focused on minimum time and relatively higher $HClO_4$ volume. Table 5 shows the modified working procedure to determine the levels of heavy metals in pasteurized milk brands.

Table 5. Modified working procedure

Modified procedure						
Acid volume (mL)		Temp (^oC)	Time (hr)	Acid %		Sample state
HNO_3	$HClO_4$			HNO_3	$HClO_4$	
3.5	2	240	3.0	69-72	70	Liquid milk

The operating conditions for FAAS employed for each analyte are given in table 6.

Table 6. Instrumental operating conditions for the determination of the metals in milk samples by using FAAS.

No	Metal	Wave length (nm)	Instrumental detection limit (mg/L)	Slit width (nm)	Lamp current (mA)
1	Zn	213.9	0.012	0.5	2
2	Cu	324.8	0.035	1.2	2
3	Cd	228.8	0.012	1.2	2
4	Cr	357.9	0.05	0.2	4
5	Ni	232	0.07	0.2	3
6	Pb	283.3	0.3	1.2	2

4.3. Precision and Accuracy

The precision of an analytical procedure describes the closeness among series of measurements. It is usually expressed as variance, standard deviation or percent relative standard deviation of a set of measurements [27].

For this study, the precision of the obtained results were evaluated by the standard deviation and percent relative standard deviation of the six samples (n=6) and triplicate readings for each sample and the results of the analysis are reported with the corresponding SD and % RSD for fat content determination. Table 11 shows the SD and % RSD of the fat content in each milk brands.

4.4. Recovery test

The recovery value for the milk samples are given in table 7. The table shows that the recovery result for zinc metal is 98.87% which is within the acceptable range (85-103%) [65]. Thus good recovery was obtained for zinc metal in Etete milk brand.

The percentage recovery of the metals was calculated by using the following equation [5, 7]:

$$\%\ \text{Recovery} = \frac{C(\text{spiked}) - C(\text{Unspiked})}{C(\text{added})} \text{x}100\%$$

Where: C(spiked) is the concentration of metal in spiked sample (mg/L).

C(unspiked) is the concentration of metal in unspiked sample (mg/L).

C(added) is amount of metal added (mg/L).

Table 7. Recovery test for zinc metal for analyzed milk samples.

Metal	Sample ID	Sample (mg/L)	[a] X ± SD	Amount added (mg/L)	Spiked (mg/L)	[b] X ± SD	% Recovery
Zn	Ete 1	0.0839	0.0988 ± 0.0220	0.0500	0.1330	0.1482 ± 0.0236	98.87
	Ete 2	0.1242			0.1754		
	Ete 3	0.0884			0.1362		

[a] mean unspiked

[b] mean spiked

4.5. Determination of the concentration of heavy metals in the milk samples

The concentration of metals in mg/g present in milk samples was calculated using the equation [58]:

$$C_{final} = \frac{CAAS \times Vs}{Ms}$$

Where,

C_{final} = Total metal concentration in liquid milk sample (mg/L).

CAAS = Concentration of metal obtained from AAS (mg/L).

Vs = Final volume of the digested sample solution (mL).

Ms = mass of sample measured during digestion (g).

Based on the above equation, the concentration of trace heavy metal in milk samples was calculated and shown in table 8. The concentration of heavy metals other than zinc in the milk brands are below the detection limit (BDL) of the instrument.

Table 8. Average concentration of zinc metal in each milk brand (µg/g)

Metal	Milk Brands					
	Shola	Family	Harme	Holland	Mama	Etete
Zn (µg/g)	2.26 ± 0.22	3.31 ± 0.41	2.12 ± 0.69	2.42 ± 0.73	1.93 ± 0.38	3.31 ± 0.67

The concentration of the metals that are below detection limits other than zinc in all milk brands can be attributed to the less contamination of the raw milk with the heavy metals collected from milk suppliers. The metals might have been removed using nano sized metal oxides during processing as the raw milk passes many processing stages.

4.6. Comparison of the concentration of metals in the different milk brands with literatures

Despite the absence of trace heavy metals in pasteurized milk brands in this study (except zinc), the metals are present in unprocessed raw cow's milk in different countries. Table 9 shows the metal content of trace metals present in cow's raw and pasteurized milk in the literatures in different countries and in the present study.

Table 9. Average metal content of cow's raw and pasteurized milk in different countries (μg/g).

Countries	Zn	Cu	Cd	Cr	Ni	Pb	References
Egypt(raw)	3.15	0.142	0.086	0.03	0.004	0.066	[19, 49]
Egypt (raw)	3.59	0.170	0.025	0.03	0.036	0.030	[66]
Pakistan (raw)	3.14	0.141	0.076	0.03	0.130	[d]BDL	[16]
USA (raw)	2.34	0.019	0.010	0.03	[c] NR	0.014	[67]
India (raw)	2.89	0.039	0.001	0.04	[c] NR	0.002	[67]
Germany (raw)	3.39	0.037	0.001	[c] NR	[c] NR	0.002	[67]
Spain (raw)	1.42	0.051	[c] NR	0.03	[c] NR	0.009	[67]
Japan (raw)	3.00	0.100	0.00	[c] NR	[c] NR	0.050	[67]
Ethiopia (raw)	5.59	0.109	[c] NR	0.87	[c] NR	[c] NR	[5]
Egypt (pasteurized)	3.11 ± 0.66	0.151 ±0.08	0.020 ±0.02	0.032 ±0.02	0.030 ±0.02	0.021 ±0.02	[66]
Ethiopia (pasteurized)	Etete = 3.31 ± 0.671	[d]BDL	[d]BDL	[d]BDL	[d]BDL	[d]BDL	Present study
	Family = 3.31 ± 0.408						
	Harme =2.12 ± 0.692						
	Holland= 2.42 ± 0.731						
	Mama =1.93 ± 0.382						
	Shola =2.26 ± 0.224						

[c] Not Reported

[d] Below Detection Limit

In the present study, Zn metal concentration in pasteurized Etete and Family milk brands are in good agreement with the literature stated in Egypt. The concentrations of zinc in Harme, Holland and Shola milk brands are slightly lower and Mama brand is relatively much lower than the literature found in Egypt.

The obtained Zn concentration in different pasteurized milk brands was also compared with the cows' raw milk in different countries and hence there is a good agreement of Etete and Family

milk brands with results found in Egypt, Pakistan, Germany and Japan. Harme, Holland and Shola Zn metal levels are in good agreement with results found in USA and India while Mama brand is slightly higher than the result obtained in Spain but lower than the literature found in all other mentioned countries. The Zn levels in the six pasteurized milk brands are much lower than its level in raw milk in the literature found in Ethiopia. Zinc concentration is found to be below the maximum permissible limit set by EFSA as the acceptable limit for human consumption of zinc is 5 mg/L [68]. The concentration of zinc metal in all milk brands is shown in figure 5.

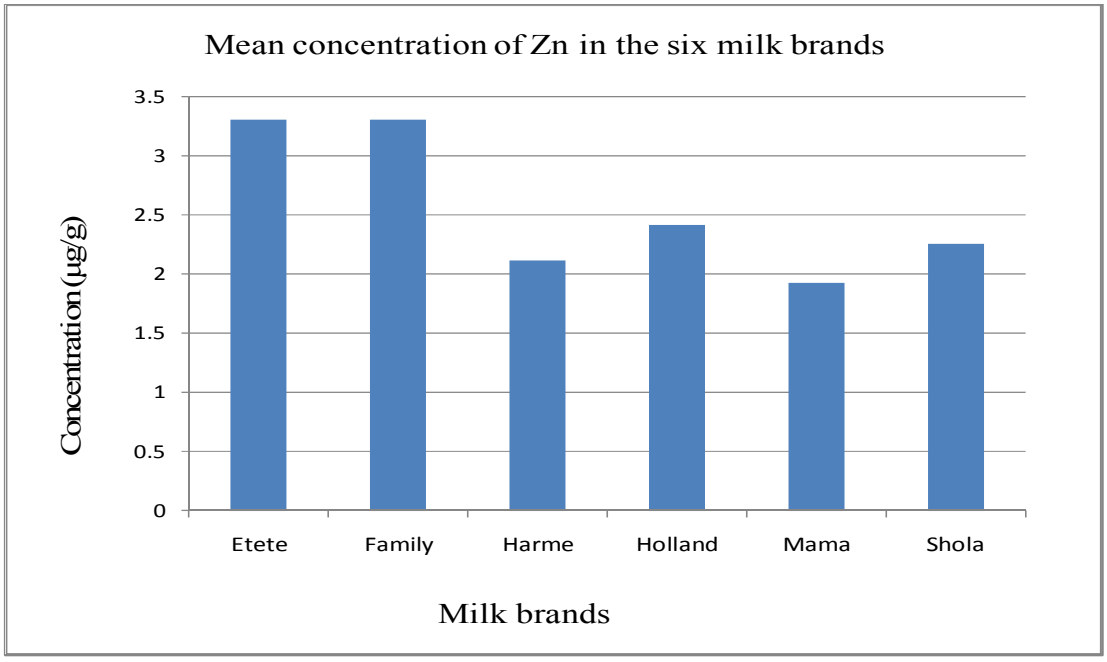

Figure 5. The mean concentration of zinc (µg/g) for each milk brand.

World Health Organization (WHO) guidelines for drinking water quality set up in Geneva, 1993, are the international reference point for standard setting and drinking water safety. Table 10 shows the maximum permissible limit of metals in drinking water set by world health organization (WHO).

Table 10. Maximum permissible limit of metals in drinking water by WHO (µg/g) [69].

Element	Health based guideline
Cd	0.003
Cr	0.05
Cu	2
Pb	0.01
Ni	0.02

4.7. Determination of fat content of the different milk brands

Accurate determination of fat in certain foods is difficult due to the binding of the fat by the matrix [63]. However the fat contents of the six milk brands were determined using the procedure mentioned. Accordingly, Mama pasteurized milk contained significantly more fat compared to other milk brands and the least is Etete milk brand. Their fat content ranges 0.12 g to 0.15 g per five gram of pasteurized milk. The fat content in gram of different milk brands are shown in table 11.

Table 11. Fat contents in the six different milk brands (per 5g of milk)

Milk brand	Sample ID	Mass (g)	Mean (g)	Standard deviation	% RSD
Etete	Ete 1	0.10	0.12	0.01	8.3
	Ete 2	0.12			
	Ete 3	0.13			
Family	Fam 1	0.14	0.14	0	0
	Fam 2	0.14			
	Fam 3	0.14			
Shola	Sho 1	0.17	0.14	0.02	14
	Sho 2	0.12			
	Sho 3	0.13			
Harme	Har 1	0.15	0.14	0.01	7.1
	Har 2	0.14			
	Har 3	0.14			
Holland	Holla 1	0.14	0.13	0.01	7.7
	Holla 2	0.12			
	Holla 3	0.14			
Mama	Mam 1	0.14	0.15	0.01	6.7
	Mam 2	0.15			
	Mam 3	0.16			

The percentage content of fat in each milk brand can be calculated as [64]:

$$\% \text{ Fat} = \frac{W2 - W1}{M} \text{ x } 100 \textbf{ \%}$$

Where: W1 is weight of empty conical flask

W2 is weight of conical flask and fat

M is mass of sample taken for test

Based on the above equation, the percentage of fat content in different milk brands was calculated and shown in table 12 and compared with the industrially determined value.

Table 12. Comparison of percentage of fat contents between the present study and on the plastic bag in the six milk brands.

	Milk Brands					
	Etete	Family	Shola	Harme	Holland	Mama
Percentage in this study	2.4	2.8	2.8	2.8	2.6	3
Percentage on plastic bag	2.8	2.7	2.7	2.8	2.7	2.7

4.8. Comparison of fat content of the different milk brands

The percentage of fat content of each milk brand is indicated on their plastic bag as 2.7, 2.7, 2.7, 2.8, 2.7 and 2.8 for Mama, Family, Holland, Etete, Shola and Harme, respectively. Similarly, the fat content of each milk brand was determined in this study. Accordingly, the fat content is in the order of: Mama (3%) > Family (2.8) = Shola (2.8%) = Harme (2.8%) > Holland (2.6) > Etete (2.4%). As can be clearly seen, the obtained results are closer to the already determined value in the factory except Etete whose determined value is slightly lower than the plastic bag value. Figure 6 shows the fat percentage of each milk brand determined in this study.

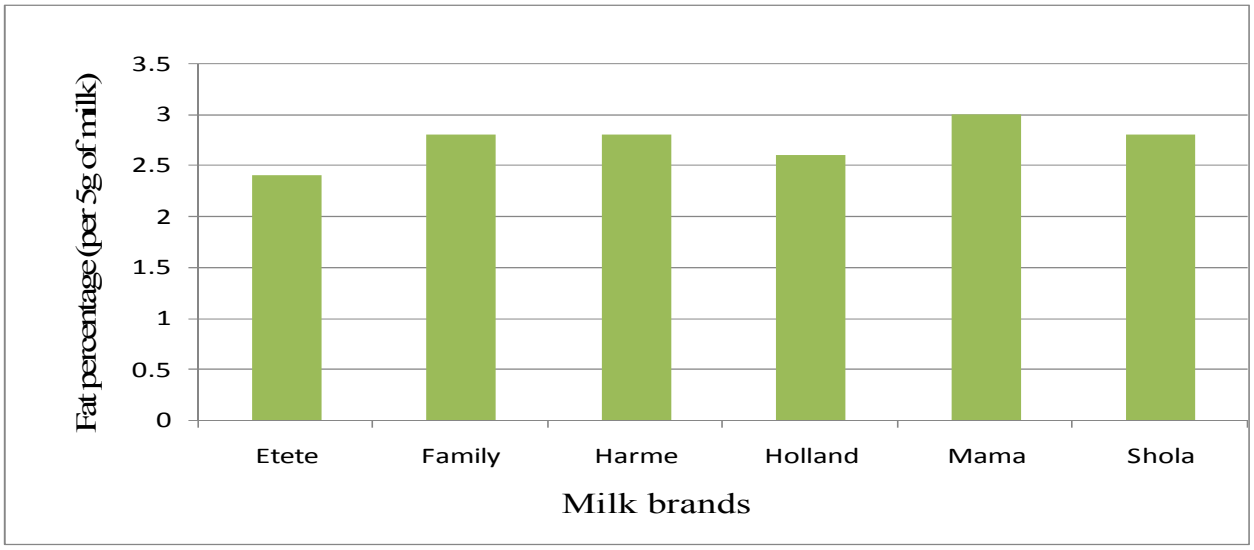

Figure 6. Fat percentage of each milk brand.

4.9. Statistical Analysis of Variance (ANOVA)

In analytical work there are often more than two means to be compared. For this case ANOVA is used to identify the source of variation of the obtained means for different experiments. As a result of variation of means from one sample to another it is used to check whether there is a significant difference or not between samples mean. The levels of significance were evaluated using P-value, where P- value less than 0.05 ($p < 0.05$) at 95% confidence level indicates there is a significant difference in means between the compared groups and vice versa.

For this study, the significance of variation between samples has been investigated using ANOVA that has been done easily on computer using Microsoft Excel 2007 software to calculate the presence or absence of significant differences in the mean concentration of the metal and fat content between six commercially available milk brands and the following results were obtained.

For Zn, no significant difference at 95% confidence level ($p > 0.05$) was observed in the mean concentrations among the six milk brands. The mean concentrations of Zn do not differ significantly ($p>0.05$) for Etete and Family, Etete and Shola, Etete and Holland, Etete and Harme, Family and Holland, Family and Harme, Shola and Mama, Mama and Holland, Holland and Harme, Shola and Holland, Shola and Harme, Mama and Harme, while it differs significantly for Etete and Mama, Family and shola, Family and Mama. This variation in concentration can be due to the difference in the source of raw milk supplied by the milk suppliers and the processing condition.

For fat content, no significant difference at 95% confidence interval ($P > 0.05$) was observed in the mean value among the milk brands. The mean fat contents do not differ significantly ($p > 0.05$) for Etete and Family, Etete and Holland, Family and Mama, Etete and Shola, Family and Holland, Family and Shola, Family and Harme, Shola and Mama, Shola and Holland, Mama and Harme, Holland and Harme. The fat contents differ significantly ($P < 0.05$) between Etete and Mama and Etete and Harme.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The concentration of heavy metals (Zn, Cu, Cr, Cd, Pb and Ni) of commercially available different milk brands which are found in Addis Ababa city, Ethiopia were determined using FAAS. The concentration of essential metal, zinc was found in the ranges of 1.93 to 3.31µg/g in all brands, which is below the set by EFSA, where as the other heavy metals were found to be below the detection limit of the instrument. The ANOVA result showed that there was no significant variation in the zinc content of each milk brands ($p > 0.05$). The efficiency of the modified digestion procedure for this study was checked by the recovery test and good percentage recovery was obtained for zinc metal. The percentage recovery test for zinc in this study is 98.87 % which is in the acceptable range stated in literatures.

The fat content of each milk brand was also determined and was found to be 2.4% to 3%. The ANOVA result also showed that there was no significant variation in the mean fat contents of all milk brands.

5.2. Recommendations

Based on the levels of the heavy metals and the fat contents determined in different pasteurized milk brands, the following recommendations can made:

1. The levels of the mentioned heavy metals were determined using Flame Atomic Absorption Spectrometry. Except zinc the rest were below detection limit. In addition to FAAS other highly sensitive instruments like Atomic Emission Spectrometer (AES), Inductively Coupled Plasma Mass Spectrometer (ICP-MS) and Graphite Furnace Atomic Absorption Spectrometer (GFAAS) could be used to check the levels of these metals.
2. As zinc is an essential element and also found below the maximum allowed limit set by EFSA, consumers can use these milk brands for their regular consumption.
3. Monitoring of the levels of toxic metals even essential metals above their maximum permissible limit should be encouraged.
4. If the milk brands under examination contain heavy metals and exceed the maximum permissible limit, nano sized metal oxides like titanium dioxide and others must be fitted to the industry to minimize their concentration.

5. Finally, the researcher strongly believes that the findings of this study can pave the way for other researchers to conduct further studies.

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