College of Natural and Computational Sciences

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Evaluation of Physicochemical properties, microbial quality, heavy metals contamination and percentage of raw materials of milk drinks in Addis Ababa

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Approval sheet

Addis Ababa University College of Natural and Computational Sciences Center for Food Science and Nutrition

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DECLARATION

This is to certify that this master's thesis entitled: Evaluation of Physicochemical properties, microbial quality, heavy metals contamination and percentage of raw materials of milk drinks in Addis Ababa, a cross sectional study was conducted by Banchayhu Getahun, and Submitted to the Center for Food Science and Nutrition for partial fulfillment of the requirements for the degree of Master of science in Food Science and Nutrition complies with the regulation of the University and meet acceptance standards with respect to originality and quality

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The thesis has been submitted with my approval as thesis advisor.

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Tables of Contents

List of tables

List of figures

List of Abbreviation

BA……Brand A

BB……Brand B

CC……...Coli form Count

CFU…… Colony-forming unit

EFDA…….. Ethiopian Food and Drug Authority

EPHI…….Ethiopian Public Health Institute

FAO…….....Food and Agriculture Organization

ISA……Institute of Ethiopian standard

MP-AES……Microwave plasma atomic emission spectroscopy

NMKL……...Nordic Committee on Food Analysis

NRV……..Nutrient Reference Value

PCA……...Plate Count Agar

QAS……..Quality assurance and certification scheme

RDI……...Reference Daily Intake

SNF……...Solid non fat

SPC……..Standard plat count

TBC…….....Total Bacterial Count

TPC…….Total plat count

TS……Total solid

UHT……Ultra High Temperature

List of Appendix s Page No

Abstract

Milk and milk products are nutrient rich foods. But, it becomes a health risk to the consumers if not handled properly due to high perishability and vulnerability to microorganisms. The present study is primarily aimed to evaluate physicochemical properties, microbial quality and heavy metals contamination of milk drinks and to determine percentage of raw materials (milk and water) to make it a protein, calcium and vitamin B12 source. A cross sectional study was conducted in all sub city of Addis Ababa and purposive sampling was conducted to collect two milk drink brands from each sub city. A total of 88 milk drink samples were collected in the study. The samples were analyzed by Lacto scan, NMKL method, MP-AES, and using Codex Guide lines. All of the physical properties and chemical composition of the milk drinks was significantly different in milk drink brands at ($p<0.05$) and were not significantly different in milk drink flavor variation at ($p>0.05$). Heavy metals: Cr and Pb was significantly different in milk drink brands at ($p<0.05$) and were not significantly different in milk drink flavor variation at ($p>0.05$). Physical properties of milk drinks significantly highest pH and specific gravity was observed in brand A milk drinks (4.22±0.05 and 1.031±0.86) respectively than in brand B milk drinks(3.95± 0.05 and1.012±0.36) respectively. The mean value of highest fat content was observed in brand B milk drinks (1.13±0.12%) than in brand A milk drinks (0.50 ± 0.11%). The highest TPC and TCC was found in brand A milk drinks (1.48 log 10 CFU/ mL and 0.548 log 10 CFU/ mL) respectively than in brand B milk drinks (1.31 log 10 CFU/ mL and 0.33 log 10 CFU/ mL) respectively. While E.coli should not be detected in heat treated foods, E.coli was detected in 4 out of 88 (4.55%) samples. None of the samples found to be positive for S. aureus, salmonella spp, Yeast and mould. The mean value of Heavy metals: highest Cr and Cd contamination was observed in brand A milk drinks (0.17±0.08 and 1.16± 0.73 mg/L) respectively than in brand B milk drinks (0.09± 0.03 and 1.02±0.39 mg/L) respectively. Similarly the highest Pb contamination was observed in brand B milk drinks (0.56±0.12 mg/L) than in brand A milk drinks (0.45± 0.15 mg/L). All pH and specific gravity of the milk drinks had not fulfilled the Ethiopian whole cow milk requirement except brand A specific gravity of milk drinks. Chemical compositions of the milk drinks had fulfilled the Ethiopian milk drink Standards except brand A fat milk drink. The microbial quality of TPC, TCC and E.coli and heavy metals, Pb and Cd of milk drinks was not complaining Ethiopian milk drink Standards. For the milk drink to be claimed as a source of protein, Ca & vitamin B12, it should be made with a minimum of 82 % whole milk add remaining could be water. Generally speaking the milk drinks were found to be not fulfilled the requirement of Ethiopian cow milk and Ethiopian milk drink standard. From this result it is possible to recommend that to ensure safety and quality of milk drinks, it is suggested to follow scientific justification behind the formulation of milk drinks and there should be a hygienic practice on milk drink production and handling.

Keywords: Milk drink; Microbial quality; raw material

1. INTRODUCTION

1.1. Background

Drinks are typically made from a range of plant and animal-based ingredients, such as fruits, vegetables, cereal grains, and milk from mammalian glands (Bolarinwa *et al*., 2018). There are several popular, widely-sold, and consumed fruit drinks and vegetable juices made from fruit, vegetables, and other natural ingredients (Bolarinwa *et al*., 2018). Drinks can increase nutrient intake and hydration (Gutierrez *et al*., 2022). Drinks are consumed with food as lunch in some countries and are used to quench thirst (Bolarinwa *et al*., 2018). Milk based drinks are the most acceptable food items for consumers in the functional food market (Hossin *et al*., 2021).

Milk is a very nutrient-dense food that provides both the macro and micronutrients required for human growth, development, and health maintenance (Zebib and Zewdu, 2020). It is primarily an excellent source of protein, fat, carbohydrates, vitamins, and minerals: calcium, magnesium, sodium, potassium, and inorganic phosphate, citrate, are among the minerals. Milk contains several different kinds of vitamins. Vitamins A, D, B12, and riboflavin are common (Ayza and Yilma, 2014) and (Tabassum and Uddin, 2016).

Ethiopia has 10 million dairy cows, and they produce 3.2 billion liters of milk per year, or 1.54 liters per cow, each day, for a total of 180 days of lactation (Getabalew *et al.,* 2019). In order to produce with a longer shelf life, milk can be processed in to different forms. In many countries, the consumption of milk has decreased in recent years (Sousa and Kopf, 2017). There is some evidence that having access to cash can give some consumer groups the freedom to buy high-quality food that they would not otherwise have access, to enhancing the nutritious value of their diets (Lemma *et al.,* 2017**).** Low milk production results from a lack of feed availability and other factors related to the rainfall pattern (Getabalew *et al.,* 2019). Many people in low and middle-income countries suffer from micro nutrient deficiencies. An important factors contributing to these deficiencies is the conception of mainly plant- based diet that are low in micro nutrients (Lemma *et al.,* 2017). Regarding the significance of milk and dairy products, modifications in food choice determinants and consumption patterns (Górska-Warsewicz *et al.,* 2019). To provide certain nutrition and to meet the linkage between demand and supply of milk, milk drinks are a simple alternative to those who only use limited variety of dietary options.

Milk drink is a drink obtained by mixing fermented or plain milk with potable water, along with or without other nondairy ingredients and flavorings (Nnubia *et al*., 2020). This is especially true for the poor and those in developing country with a limited variety of dietary options. Commercial milk drinks are those that are made for consumers as ready-to-drink beverages (Nnubia *et al*., 2020). Consumers who are concerned about their health show a lot of interest in low-calorie milk and milk products. It supplies water and energy for the body (Kirdat *et al*., 2019).

Currently, healthy eating is encouraged as a method of preventing deficiencies diseases brought on by inadequate nutritional consumption (Nnubia *et al*., 2020). Consumers desire food that is healthy, wholesome, and nutrient-dense, as well as food that has been produced and prepared in a safe, sanitary manner and is pathogen-free. Quality dairy products are those that are without of dangerous poisonous chemicals and pathogenic microbes (Ayza and Yilma, 2014).

Bacterial pathogens transmitted to humans include *salmonellosis*, *staphylococcus aures*, *Escherichia coli* and extra (Hahn, 1996). Milk's chemical composition results high moisture content, pH that is almost neutral, and high vitamin content may create a positive atmosphere of bacteria, yeast and molds in the milk to survive, grow and reproduce even though they are pasteurized or refrigerated (Jamal *et al*., 2018). After pasteurization, milk can also become contaminated because Bacteria have the ability to produce heat-stable proteases and lipases, which are still active after pasteurization (Fusco *et al*., 2020).

It was also mentioned that pasteurized milk may be contaminated as a result of poor bacteriological quality of milk and insufficient plant cleanliness (Saha and Ara, 2012). The causes of the high bacteria count in the pasteurized milks may include defective pasteurization machinery, surviving pasteurization, and post-pasteurized contamination as a result of poor processing and handling conditions and/or poor hygienic practices by the concerned employees (Saha and Ara, 2012).

Milk and its products are the most commonly consumed foods by people, particularly by children, it is critical to look into the presence and quantity of hazardous heavy metals in milk samples **(**Abdeljalil *et al*., 2021**).** Heavy metals are a group of metals and semimetals (metalloids) that have been linked to pollution and possible toxicity. Heavy metals are dangerous

at large amounts because they interfere with regular metabolic processes (Abdeljalil *et al*., 2021). Heavy metal contamination in milk and dairy can be caused by raw materials from milking due to the contamination of milking animals or by machinery and equipment in contact with dairy during production and storage. Metals including copper, zinc, iron, tin, lead, arsenic, and cadmium are the primary components of metallic impurities in metal containers used to preserve milk during technological processes **(**Islamoğlu *et al*., 2020**).**

As far as our knowledge in the area is concerned, there are no or little studies on milk drinks that could be due to the fact that milk drink is a relatively recent product, especially to Ethiopia. Therefore, this study aims at investigating the physicochemical properties, microbial quality and heavy metals contamination of milk drinks sold in Addis Ababa city, Ethiopia. The study also focused on determining the percentage of the main raw materials (water and milk) of the milk drinks to make it as a source for some of the nutrients (protein, calcium, and vitamin B12).

1.2. Statement of the problem

One of the characteristics of malnutrition that is currently of concern to the world's public health is under nutrition, particularly in young children. These results in illnesses that have the potential to stunt children's growth and development, and it may also cause cognitive decline and poor academic performance in growing children (Lemma *et al*., 2017). Micronutrient deficiencies affect a lot of people in low- and middle-income countries (Lemma *et al*., 2017). In developing countries, between 20 to 80 percent of schoolchildren suffer from inadequate nutrition and low nutrient intake, which is linked to 28 percent of child deaths and morbidities (Nnubia *et al*., 2020).

In Ethiopia, there are serious concerns about milk drinks' nutritional value because so far there is no scientific justification behind the formulation of milk drinks. This creates a concern that the milk drinks available in the market may not provide nutrients to the level that satisfies the need of the consumers and the recommendation of global organizations including Codex Alimentarius. This situation may aggravate the already existing nutritional deficiencies in the country.

The chemical character of whole milk creates conducive environment of the milk drinks (high moisture, neutral pH and nutrients) and addition of further water to the milk during milk drink preparation may also favors the growth of the pathogenic microorganisms. Addition of water not only favoring microbial growth, it could have an impact on the nutrient content of the milk by diluting the contents. If pathogenic bacteria are present in milk drinks, users could face serious health risks and it could also probably cause death (Dey and Karim, 2013). Moreover, addition of plain water to the milk during milk drink preparation may also increase the contamination of heavy metals.

So this study was conducted to evaluate physicochemical properties microbial quality, heavy metals contamination of milk drinks sold in Addis Ababa city and to determine percentage of raw materials (milk and water) to make it a protein, calcium and vitamin B12 source.

1.3. Objective

1.3.1. General objective

To evaluate physicochemical properties microbial quality, heavy metals contamination of milk drinks sold in Addis Ababa city and to determine percentage of raw materials (milk and water) to make it a protein, calcium and vitamin B12 source.

1.3.2 Specific objectives

- To evaluate nutritional content of milk drink
- To evaluate the microbial quality of milk drink
- To determine the heavy metal contaminants of milk drinks
- To evaluate the product level of physicochemical and microbial compliance with the existing Ethiopian milk drink specification
- To evaluate the percentage of the main raw materials (milk and water) of milk drink to make it source of protein, calcium and vitamin B12

1.5 Significance of the study

- Results obtained from this current study can safe guard the health of the consumer by increasing knowledge of physicochemical properties and microbial qualities of commercial milk drinks**.**
- The research will promote awareness to milk drink processors or offer them with important information on percentage of the main raw materials to be used to make the product a source of protein, calcium and vitamin B12
- Provide information to the Ethiopian regulatory bodies they could use it as an input during revising the requirements of the Ethiopian Milk drink specification

2. Literature review

2.1. Drink/Beverage

Beverages are typically made from a wide range of plant and animal-derived raw materials, such as fruits, vegetables, cereal grains, and milk from animal mammary glands (Bolarinwa *et al*., 2018). In actuality, there is a wide variety of products with varying percentages of fruit juice. These beverages may be purchased in a ready-to-drink version or as 'cordial,' sometimes known as dilutable liquids (Caswell, 2009). A variety of fruit drinks and vegetable juices made from fruit juices and vegetables, as well as other natural components, are popular, sold, and consumed around the world (Bolarinwa *et al*., 2018). According to estimates Fluid milk accounts for between 60 and 80 percent of the dairy products consumed by American children (Nnubia *et al*., 2020). Fluid milk category includes plain and flavored milk as well as milk drinks (Sebastian *et al*., 2010).

Milk based Drinks are generally the beverages typically have liquid milk or skim milk powder as the primary ingredient. The main ingredients used in the production of these drinks include skim milk, sugar, preservatives, colors, flavors, acids, beneficial additions, fruit mixes, juices, and concentrates, along with water (Mudgil and Barak, 2019). Drinks are convenient diets that are well-liked for their ability to quench thirst and refreshing properties. Additionally, some beverages can give users energy and other minerals (Bolarinwa *et al*., 2018). Despite the remarkable increase in the production of ready drinks in recent years, there are more potential demands for healthy and useful beverages (Abbasi and Mohammadi, 2013). To give the body all the nutrients it needs to be healthy, it involves consuming a variety of nutrient-dense meals, like milk and its byproducts (Nnubia *et al*., 2020). It indicates that milk is the primary dairy product consumed by children worldwide (Mahato *et al*., 2020). They provide proteins, vitamins, and minerals to consumers (Mudgil and Barak, 2019). Due to the nutrients they contain, some beverages can meet a human's nutritional gap, while others are eaten as stimulants (Bolarinwa *et al*., 2018).

2.2. Milk production in Ethiopia

In Ethiopia, there are three categories for dairy production systems: rural, peri-urban, and urban. Producers of mixed crop livestock as well as pastoralists and agro pastoralists make up the

supply of the rural dairy small-holder system. Producers of livestock account for the majority of the milk produced, producing 98% of it (Gutema, 2022). Ethiopia has Africa's greatest population of livestock (Yilma *et al*., 2011) which has populations of 31.3 million sheep, 60.3 million cattle, and 32.7 million goats. In Ethiopia, there are about 10 million dairy cows that produce 3.2 billion liters of milk annually on average at 1.54 liters per cow per day during a 180-day lactation period. An estimated 32% of the milk produced is used by calves and wasted (Getabalew *et al*., 2019).

The primary causes of the low productivity of the country's livestock production system and particularly in the traditional sector, there are the lack of crossbred dairy cows, the lack of capital for dairy producers, inadequate animal feed resources in terms of quality and quantity, inadequate animal husbandry systems, ineffective and inadequate milk processing materials and methods, low milk production and supply to milk processing centers, and poor marketing and market conditions (Yilma *et al*., 2011). Due to inadequate adoption of better breeds and poor management techniques, milk yields on the production side are only a small portion of their potential (Hoddinott *et al*., 2015).

2.3. Milk consumption in Ethiopia

Rural households produce more milk than they can consume in their own homes by over 85%, with fewer than 7% of that milk being sold. Ethiopia's low marketable yield decrease its vast number of milk producers producing a small amount of milk (Abebe *et al*., 2013). The milk that pastoral children drink during the rainy season can supply 100% of their protein needs and 67% of their average daily energy needs. Milk consumption declined daily during the dry season by over 25% due to access and availability issues, with milk only meeting 16% and 50% of the region protein and calorie needs, respectively. Children's milk consumption will typically decrease by 50% during the dry season (Getabalew *et al*., 2019). Over two million people are thought to be suffering from chronic food insecurity, Ahmed, 2004 said that according to the FAO's assessment that about 51% of the population is undernourished. Ethiopians consume fewer dairy products than people in other African nations. Due to poor pre-milking and post-harvest handling procedures, a high degree of milk perish ability, and poor pre-milking techniques, Ethiopian milk production falls below of international standards. Poor personal hygiene of the milkers, disregard for the hygienic state of the milking environment and milk containers, a lack of udder and teat cleaning procedures, a failure to use towels for udder

washing and drying, and poor milk quality due to microbial contamination are all factors that contribute to poor and substandard milk production in Ethiopia. Per capita intake of milk in Ethiopia is as low as 17 litter per head (Ahmed *et al*. 2004) and according to Gutema, 2022 In Ethiopia each person consumes about 19 litter of milk annually (Gutema, 2022).while the average figure for Africa is 26 litter per head (Ahmed *et al*. 2004).

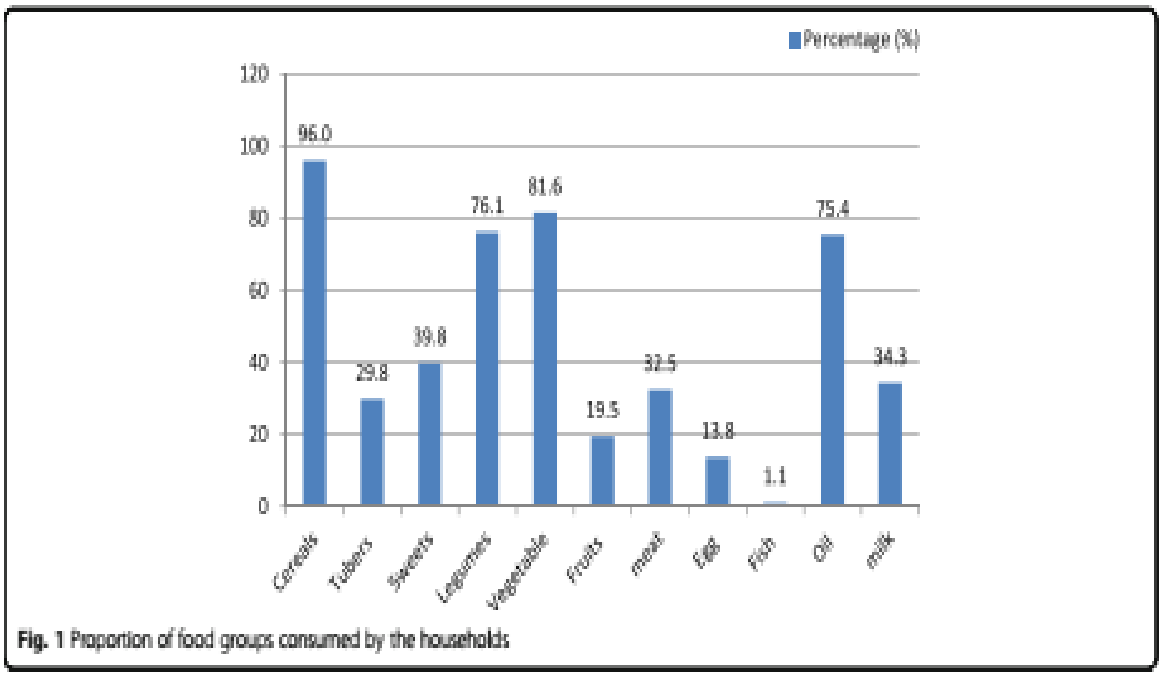


Figure 1.Proportion of food groups consumed by the households (Workicho et al., 2016)

2. 4. Nutrition and Health benefit of milk

Food insecurity, poverty, and inadequate nutrition are continual issues in many developing nations (Pelletier *et al*., 1995). Africans' diets have always been heavily reliant on milk and dairy products. They still have a significant and increasing impact on the diets of the rising numbers of people living in both rural and urban areas (Dirar, 1993). The world's most consumed milk is cow milk, followed by goat, camel, and donkey milk (Getaneh, 2022). It is the most widely consumed food for humans and is regarded as a complete and nutritious food for all age groups in both rural and urban populations around the world. Milk and dairy products include a number of essential nutrients for the growth and maintenance of the human body (Hossin *et al*., 2021).

From birth, milk is an essential component of a healthy human diet. Milk and dairy products include a variety of necessary nutrients for the growth and maintenance of the human body (Kailasapathy, 2015). This includes macronutrients and micronutrients such as lipids, proteins,

carbohydrates, vitamins, minerals, and active substances that play a protective effect for one's health (Soliman, 2005) as well as riboflavin, vitamin D, calcium, phosphorus, and prebiotics (Kailasapathy, 2015). Protein, fat, and lactose nutrients included in milk constitute a significant source of energy (Getaneh, 2022). Milk contains the majority of the minerals that humans need. It mostly comprises calcium and phosphorus and is the main source of bioavailable calcium in our diet (Soliman, 2005). Milk helps children grow by giving them the nutrients they need to stay healthy, keep their bones strong, and develop normally (Kailasapathy, 2015). Calcium each milliliter of regular milk provides about 1.2 milligrams of calcium. In a moderate serving of the product, the majority of milk and dairy products can provide 100% of the RDI for calcium. Calcium from dairy products promotes strong bones and teeth, as well as kidney stones, colon cancer, and infectious disorders. Dairy components are therefore a crucial component for public health nutrition (Kailasapathy, 2015). Bovine milk has long been one of the most significant food sources in the human diet since it is a vast reservoir of various nutrients and bioactive substances (Kopf-Bolanz *et al*., 2012).

These nutrients are crucial for bone development, bone health, and total nutrient sufficiency in both childhood and adulthood. It contributes to bettering diet quality and bone health. It aids in lowering the risk of osteoporosis. It aids in lowering the danger of hypertension. It lowers the danger of becoming obese. It lowers the danger of developing metabolic syndrome (Praveen Kumar *et al*., 2019) and (Akbay and Tiryaki, 2008). The ideal milk composition is suitable for both adults' energy needs as well as those of growing children (Hingne and Chavan, 2021)

2.5. Physicochemical characteristics of Milk

The physical attributes and chemical makeup of milk served as indicators of hygienic standards compliance. The protein, fat, lactose, SNF, TS, vitamin and mineral concentrations, and ash content were the chemical characteristics of milk. The pH, acidity, freezing point, and specific gravity of milk were its physical characteristics. These parameters provided information about the nutritional value and standard of milk (Getaneh, 2022).

2.5.1. Chemical properties of milk

Milk long been considered as a healthy food. Milk provides essential elements such as proteins, carbohydrates, vitamins, minerals, and lipids (Hingne and Chavan, 2021)

Water

All of the other components of milk (total solids) are suspended or dissolved in water. (Kailasapathy, 2015) Water is the nutrient that all animals need in the highest concentration, and milk has high water content (88.6%). The amount of lactose produced by the mammary glands secretor cells regulates this quantity of water (Guetouache *et al*., 2014). Some of the water in milk is chemically or physically hydrated into lactose, salts, and proteins. By lowering water activity, milk's shelf life is increased when water is removed, as in concentrated and dried milk products (Kailasapathy, 2015).

Lactose

The primary carbohydrate in milk is lactose (Kopf-Bolanz *et al*., 2012). It is composed of one D-galactose molecule and one D-glucose molecule. Lactose is not sweet tasting even though it is a sugar. It is utilized as a substrate by lactic acid bacteria during the fermentation of milk, and the amount of it varies somewhat in milk (4.5 to 5.2 g/100 g), but not in fermented foods like cheese and yoghurt. It aids in the process of fermenting milk (Guetouache *et al*., 2014).

Milk Proteins

The amino acids needed for a baby or child's growth are provided by the protein in their food. It is also necessary for the adult tissues' maintenance (Ayza and Yilma, 2014). Milk contains a mixture of colloidal suspension and solution-based proteins. Whey protein (also known as serum proteins) and caseins are the two main forms of milk proteins. Although the proportion of whey protein to casein varies depending on the stage of lactation, casein makes up more than 80% of the protein in milk (Kailasapathy, 2015). Milk's high-quality proteins provide all the necessary amino acids as well as other components that our bodies are unable to manufacture. It's critical to keep in mind that proteins serve as the foundation for all living tissue. Indeed, the sulfur amino acids are restricting factors in milk. A considerable amount of all the amino acids required for growth and maintenance are present in casein and, even more, the complex milk protein (Guetouache *et al*., 2014).

Milk Lipids

Among all the components of milk, milk fat varies the greatest. Triglycerides make up the majority of fat (TG). Phospholipids, steroids, carotenoids, and fat-soluble vitamins A, D, E, and K make up the remaining 1%–2% of milk fat (Kailasapathy, 2015). The main source of energy in milk is fat. Milk contains fat in the form of an emulsion of fat cells; the majority of the milk's fat

content is concentrated in small cells suspended in water. Simple lipids (triglycerides) and complex lipids (phospholipids) are the two main categories of lipids in milk by providing 9 kcal/g of dietary lipids as energy milk fat serves a nutritional purpose (Guetouache *et al*., 2014). Milk fat contributes to the body's construction and gives the body vital vitamins. Vitamins A and D mentioned above; vitamin A is crucial for the epithelia, which explains its involvement in reproduction and eye sight, whereas vitamin D is crucial for binding calcium and promoting bone formation. Therefore, fat milk has a role in the diet, but it needs to be sensible: high energy value, like all lipids; saturated fatty acids and cholesterol are present, both of which are toxic in high doses. Because all lipids contain saturated fatty acids and cholesterol, which are detrimental in high amounts, milk fat has a role in the diet (Guetouache *et al*., 2014).

Minerals

They are crucial to the structural organization of casein micelles, the primary mineral found in milk, Calcium, magnesium, potassium, sodium, and bicarbonates of these minerals make up the majority of the minerals in milk (Kailasapathy, 2015). The calcium in milk is readily absorbed by the body. Phosphorus affects absorption and utilization of calcium. These two minerals are present in milk in approximately the same ratio as found in bone (Hingne and Chavan, 2021).

Vitamins

Fat-soluble and water-soluble vitamins are two main categories of vitamin in milk Vitamin E, D, and A levels can vary. Since they are fat-soluble, they can be lost via skimming and are present in fat. The serum contains additional water-soluble vitamins such as Ascorbic acid (Vitamin C), which is found in small amounts in fresh milk, is destroyed both when it comes into contact with air and when it is pasteurized (Guetouache *et al*., 2014). Milk contains a significant amount of riboflavin, or vitamin B2, in addition to vitamins A and D. Riboflavin supports healthy skin and eyes (Hingne and Chavan, 2021). Milk has a very high nutritional value because its nutrients are balanced (Guetouache *et al*., 2014).

Table 1.Typical Chemical Composition of Milk of Different Species (% Composition)

Species	Water	Fat	Protein	Lactose	Ash
Ass	90	1.3	1.7	6.5	0.5
Buffalo	84.2	6.6	3.2	5.2	0.8
Camel	86.5	3.1	4	5.6	0.6

Cow	86.5	4.6	3.4	4.9	0.5
Ewe	79.4	8.6	6.7	4.3	1.0
Goat	86.5	4.5	3.5	4.7	0.8

(Kailasapathy, 2015)

2.5.2. Physical properties of milk

Measurements of the physical properties of milk and dairy products are used in the dairy business to estimate the concentration of one or more components (e.g., specific gravity to identify added water or freezing point to assess solids-not-fat), to get information required for the design of dairy equipment (such as heat conductivity and viscosity), or to measure the degree of a physical or chemical change (such as titratable acidity to follow bacterial action or viscosity to evaluate the aggregation of fat globules or protein miscelles (Sherbon, 1988).

2.6. Milk regulatory mechanism in Ethiopia

The quality and safety of foods and food products produced domestically in Ethiopia are regulated by the Ministry of Trade and Industry, the Quality and Standards Authority of Ethiopia, the Agricultural sector, and the Health sector. To ensure the quality and safety of foods originating from plants and animals, the agricultural sector is authorized to enact laws and carry out control and inspection procedures. The safety and quality of animals, animal products, and plants are safeguarded by this action. The issues include outdated and disjointed food-related laws, as well as insufficient coordination between federal and state regulatory entities involved in the operations (Ayza and Yilma, 2014).Food safety regulations require a lot of attention as the sector expands in order to provide consumers with a product that is both safe and good quality. To implement the high standards set by the government, there has been a lack of coordination and cooperation among various regulatory authorities. Additionally, the country lacks an up-to-date comprehensive food law that outlines and streamlines the duties of each regulatory authority (Merwan *et al*., 2018).

2.7. Quality assurance and control of milk and milk products

A quality assurance and certification scheme (QAS) is any code of conduct, standard, or set of rules that enables food supply chain stakeholders to guarantee compliance with what is stated and to convey this to the end or future user. When it comes to a product's biochemical composition, origin and the origin of the raw materials used to make it, production methods, and

pesticide residues in the product, animal breeding and living conditions, and ethical aspects of production, QAS generally tries to differentiate and guarantee the product (Merwan *et al*., 2018).

2.8. Public health standard of milk

In many developing countries, including Ethiopia, there have been several reports of milk adulteration with water and other substances. Adulteration alters the natural composition of milk and may introduce harmful substances and pathogenic microbes. Consumption of adulterated milk can be harmful to one's health if it contains harmful ingredients or has lost nutritional value (Desye *et al.,* 2023).

Although milk is thought to be sterile prior to milking, its quality is contaminated during handling, processing, and storage. A variety of bacteria, including *Bacillus cereus, Salmonella spp., Listeria monocytogenes, Staphylococcus aureus*, and *Campylobacter spp*, can cause food-borne illnesses when they are present in contaminated milk (Desye *et al.,* 2023). Numerous factors, including hereditary ones like the breed of the cow and non-genetic ones like the stage of lactation, feeding schedule, interval between milking, and completeness of milking, can also have an impact on the composition of milk (Desye *et al.,* 2023). Dairy products for consumers must be manufactured and handled in a hygienic way, free of hazardous substances and pathogens. Thus, meeting consumer demand for quality milk depends on producing and delivering milk of high quality (Desye *et al.,* 2023). Improvement of the physicochemical characteristics of milk frequently has a major impact on consumers' health and safety (Desye *et al.,* 2023).

The effects of high microbial loads in pasteurized or UHT milk are unpredictable. For pasteurized and UHT milk, the indicated expiration dates are seven days and six months, respectively, from the date of production. However, poor preliminary milk quality, improper dispensing, or an issue with preservation on the side of the user could cause milk quality to decline before its expiration date (Jamal *et al*., 2018). Due to its extensive use by newborns, children, and adults, milk has been identified as the food for which chemical and microbiological quality control should be attained (Saha and Ara, 2012).

2.9. Milk drink

A milk drink can be made by combining fermented or plain milk with drinkable water, along with or without other nondairy ingredients and flavorings. Due to its high nutrient composition

during manufacture, it is one of the healthiest beverages available. This is true especially for the poor and those in developing nations with a limited variety of dietary options. Commercial milk drinks are those that are made as ready-to-drink beverages for consumers. Worldwide use of commercial milk beverages has increased, and dairy companies are producing new products with additional value (Nnubia *et al*., 2020).

Commercial milk drinks go through the processes of pasteurization, homogenization, and fortification. These procedures aim to increase the products' more palatable and nutritious value to the consumer in addition to extending their shelf lives. Since milk is the main ingredient in milk drinks, they must include almost all of the elements found in raw milk to be considered nutritious (Nnubia *et al*., 2020). Commercial milk products may be frequently useful in Ethiopia when individuals are looking for portable, nutritious drinks for kids' school snacks and other events. Milk drinks come in a variety of styles and brands.

2. 10. The role of milk drink standard in Ethiopia

According to the Ethiopian standards agency ESA, 2021 specification Compositional requirements and pathogenic microorganisms for milk drink. Microbiological requirements for milk drink, to mention microbiological limits set by ESA(Ref No ES6633 : 2021) the total plate Count should be 10 cfu/ml Maximum, total coli form Count, *Shigella*, Staphylococcus aureus, salmonellae spp and *E.coli* should be nil cfu /25 ml, yeast and mould should be 10 cfu/ml Maximum. ESA recommended that Milk drinks shall be free from pathogenic microorganism and shall comply with microbiological limits (ESA, 2021). Compositional requirements for milk drink as show in table 2

Table 2. Compositional requirements for milk drink

Characteristics	**Requirement**	**Test Method**
Fat, %, Min.	1.10	ES ISO 1211
Protein, %, Min.	1.00	ES 586, ES 587
Total solid not fat, Min.	2.90	ES ISO 8851-2
Probiotics (cfu/g, total), Min	10^6	ES ISO 2720

(ESA, 2021)

2.11. Microbiological quality of milk

Bacterial populations in processed milk are an indication of processing plant flaws. The presence of pathogenic organisms, high coliform counts, and high levels of adulteration in milk are warning signs of a potentially dangerous product that could seriously endanger consumers' health (Dey and Karim, 2013). It is commonly known that disease-causing microorganisms can be present in milk. Numerous reports have been made of milk-borne outbreaks (Kailasapathy, 2015). One of the most valuable and often consumed foods is milk, which is regarded as nature's most complete food. However, it is also very susceptible to bacterial contamination, making it quickly perishable. Despite having a high nutritional value, it also makes a great medium for microbial development. Pathogenic bacteria can result in serious health risks for users, including death (Dey and Karim, 2013). But customer concerns about the quality and safety of milk and dairy products, particularly for infants and young children, have grown significantly (Zebib and Zewdu, 2020). Pasteurization does not eliminate organisms that develop slowly or produce spores (Reta and Addis, 2015). The majority of spore-forming bacteria can also create biofilms, which present additional obstacle for the dairy industry because they are very resistant to temperature changes and sanitary conditions. Specific spore-forming bacteria that produce lipolytic and proteolytic enzymes under refrigeration might break down the main milk constituents, decreasing the sensory quality of the beverage (Ortuzar *et al*., 2018). High ambient temperatures would result in very poor milk keeping qualities, and there would be a very high risk of degradation if production and handling hygiene standards were poor (FAO, 1990).

The quantity and types of spoilage bacteria found in milk are considered to be indicators of milk quality. One of the most significant quality indicators that have a direct impact on milk pricing is the microbial count (Fusco *et al.,* 2020). The goal is to reduce spoiling on a variety of levels. One is to make an effort to reduce the amount of bacteria present by avoiding contamination along the entire dairy supply chain. Effective temperature control is necessary from the time of milking till raw milk is stored in the bulk tank; notably in light of the lack of chemical indicators (freezing point, protein and fat content, and absence of inhibitory substances)(Fusco *et al.,* 2020).

2.12. Source of bacterial contamination

Each dairy product contaminant is explained along with the extent to which it can make the product unsafe. These include improper handling, poor storage conditions, naturally occurring toxins found in the food, contaminated water, pesticide and drug residues, and inadequate

temperature control (Merwan *et al.,* 2018). Microorganisms can contaminate milk during the processing, transportation, storage, and preparation of milk products for consumption. Milk provides microbes with the means to proliferate quickly (Saha and Ara, 2012).

2.13. Coliform bacteria in milk after pasteurization

They are referred to as indicator organisms in science, because the presence of coliforms in food suggests a potential contamination. The Existence of coliform in pasteurized milk products suggests post-pasteurization recontamination (Dey and Karim, 2013). Raw milk is pasteurized before being marketed for consumption as liquid milk. *Micrococcus, Enterococcus*, certain *Streptococcus, Corynebacterium*, and spores of *Bacillus* and *Clostridium* are among the thermoduric bacteria that survive the procedure. Additionally, post pasteurization contaminants such as coliforms, Pseudomonas, Alcaligenes, Flavobacterium, and others can enter. Under refrigeration, pasteurized milk has a short shelf life mostly due to the growth of certain psychotropic contaminants. They exhibit the same pattern of deterioration as that of raw milk. When the population exceeds 106cells/mL, flavor abnormalities caused by their expansion can be identified (Ray and Bhunia, 2013).

The deterioration of pasteurized refrigerated milk has been linked to the growth of psychrotrophic Bacillus species, including Bacillus cereus, even in cases where post-pasteurization contamination levels are low. Spores survive pasteurization; they germinate and multiply to cause a defect known as bitty (Ray and Bhunia, 2013).

There are two types of spore-forming bacteria that are important to the dairy industry: anaerobic spore-forming bacteria like Clostridium tyrobutyricum and aerobic spore-forming bacteria like Bacillus cereus, Paenibacillus spp., and Geobacillus stearothermophilus. Aerobic spore-forming bacteria have a significant role as indicator of the quality of milk powder (mesophilic and thermophilic strains) and may limit the shelf life of fluid milk (psychrotrophic strains), depending on their growth characteristics (Ortuzar *et al*., 2018).

Escherichia coli

It is *Escherichia coli*. It is a common source of contamination and reliable indicator of fecal pollution in general in the hygienic conditions of water, food, milk, and other dairy products (Aberra, 2010). The gastrointestinal system of mammals is home to *E. coli* naturally. While the majority of *E. coli* strains are not harmful to people, some are known to be particularly

dangerous because they produce toxins or have other virulence factors. Shiga toxin-producing *E. coli* is one of the pathotypes of the bacteria that is particularly worrying (Keba *et al.,* 2020).

Salmonella

Up to 1.3 billion cases of disease are reported each year due to Salmonella enterica (S. enterica), a facultative intracellular anaerobe that is Gram-negative and important worldwide (Coburn *et al.,* 2007). Human cases of salmonellosis outbreaks linked to food in recent years have highlighted the significance of milk and milk products as potential sources of infection (d'Aoust *et al*., 1987). More than 1500 cases of salmonellosis caused by Salmonella typhimurium have been linked to cheddar cheese produced in eastern Canada from milk that was not properly pasteurised or heat-treated (d'Aoust *et al*., 1987). *Salmonellosis* in humans is known to be caused by facultative anaerobic microbes. Salmonella enterica is the species in the genus that has six subspecies, and S. enterica subspecies enterica is the one that causes 99% of infections in both humans and animals. Fruit, produce, ready-to-eat meals, chicken, beef, and other foods have all been related to salmonella outbreaks (Keba *et al.,* 2020).

Staphylococcus aures

Staphylococcus aures a common cause of mastitis in dairy cattle, can get into the milk supply through the cows' teat spots or by their hands and nasal discharges. An enterotoxin is produced by the Staphylococcus bacterium when raw milk is stored at temperatures higher than fifty degrees Fahrenheit (toxins that cause vomiting and diarrhoea). People can become ill from eating enough enterotoxins in their food. Staphylococcal toxicity has been considerably reduced as a result of pasteurisation (Aberra, 2010).

Shigella

Shigella is a significant human food-borne infection that can cause clinically severe diarrhea. About 1.8 million persons have died from diarrhea, with *Shigella* being blamed for the majority of these cases (160 million every year). Studies have shown a connection between Shigella and the majority of bacillary dysentery cases in developing countries. *Shigella* is a significant bacterial pathogen that exists throughout the world. It can cause a number of illnesses, including purulent blood, diarrhea, spasms, and shocks (Zhang *et al.,* 2018).

Yeast and mold

Yeasts are classified as unicellular fungi that reproduce by budding or fission with the exception of Geotrichum candidum. Yeasts are important for dairy product fermentation, maturation, and spoiling. Probiotics made by yeasts, beneficial yeast traits for dairy products, and the possibility of using yeasts as starting cultures in the dairy sector (Jakobsen and Narvhus, 1996). However, yeasts also play a role as food and beverage spoiling organisms. The detrimental impact is associated with well-known physiological traits of yeasts, such as their capacity for low-temperature multiplication, metabolic processes, and resistance to physico-chemical stress that are crucial for food preservation. Consequently, yeast contamination must be taken seriously in a number of food and beverage processing sectors across the whole supply chain, from the raw material to the finished product. This dual role of yeasts is clearly demonstrated within the dairy industry (Jakobsen and Narvhus, 1996).

In the dairy industry, mould deterioration is a big problem that leads to a lot of food waste and big financial losses. Mould can grow easily on dairy products, and the product surface can show noticeable changes as a result of this expansion. The Penicillium and Mucor genera, two of the most prevalent moulds, are responsible for the spoiling of dairy products. Although penicillium species are mostly isolated from cheese, they can also be found in yoghurt, butter, and milk, among other products. Mucor spp. is frequently used in cheese and yoghurt to cause deterioration (Shi and Knøchel, 2021).

2.14. Pasteurization

Pasteurization is the process of heating milk to a specific temperature for a predetermined amount of time in order to kill pathogens. Bacteria are destroyed by heat at a rate that is proportionate to their quantity since the process is logarithmic. Pasteurizations eliminates pathogenic and spoilage organisms from a product, increasing product safety and extending products shelf life (Reta and Addis, 2015). The whole point of pasteurizing milk is elimination of all the bacteria that cause sickness. Pathogen endospores like Clostridium botulinum and spoilage organisms like Bacillus cereus, C. sporogenes, and Clostridium tyrobutyricum do not get eliminated. While non-thermal methods can be employed to eliminate pathogens, heating is the only way to pasteurize milk. A variety of psychrotrophic organisms belonging to the genus Bacillus are among the survivors (Jay *et al*., 2008).

Basically, the pasteurization of milk serves two different goals. This are Aspect of public health ensuring that all potentially hazardous bacteria are eliminated in order to render milk and milk products safe for human consumption and Aspects of milk and milk products' keeping quality that can be improved (Reta and Addis, 2015). If milk is not pasteurized correctly, there may be a significant microbial burden in the milk. It is advised to drink pasteurized milk within seven days of manufacturing, while UHT milk should be eaten within six months. However, poor initial milk quality, improper processing, and consumer-side preservation issues may result in microbial contamination in milk, and as a result, there are significant risks that milk will deteriorate much sooner than the advised preservation time (Dey and Karim, 2013).

2.14.1. Method of pasteurization

The batch method, low temperature-long time (LTLT) method

Consists of heating the coolest part to 145◦F (63◦C) for 30 minutes. This is referred to as the batch method (Jay *et al*., 2008).

The continuous method known as the high temperature-short time (HTST) method

The high temperature-short time (HTST) approach, which is more commonly utilized, involves heating to 161 °F (72 °C) for 15 seconds. Its inherent reduced destructiveness compared to the batch procedure makes it the flash method. The thermal death time (TDT) of the most heat-resistant non-spore forming milk-borne pathogens serves as the basis for the heating time and temperature (Jay *et al*., 2008). **UHT (ultra-high temperature);** UHT (ultra-high temperature) is another thermal treatment that eliminates non-spore forming pathogens in milk while also significantly reducing the amount of some spore formers. The UHT process involves heating at temperatures between 275-284 F (135-140 ^{0}C) for a short period of time (the minimum treatment time is 1 sec) When aseptically packaged in sterile containers, UHT-treated milk has a shelf life of 40–45 days at 40°F and is commercially sterile (Jay *et al*., 2008).

Table 3.Time and temperature for pasteurization of fluid milk (Aberra, 2010)

Temperature	Time in seconds
64 ^{0}C (145ºF)	1800
72 ^{0}C (161 ºF)	15
89 ^{0}C (191 ºF)	1
90 ^{0}C (194 ºF)	0.5

94 ^{0}C (201 ºF)	0.1
96 ^{0}C (204 ºF)	0.05
100 ^{0}C (212 ºF)	0.01

2.15 Heavy metals

Because of human activity, concentrations of some hazardous metals have significantly increased (Abdeljalil *et al*., 2021). The amount of dangerous heavy metals in the air, water, plants, and soil might increase due to growing industrial, automotive, and agricultural processes (Asayehegn et al., 2021). Heavy metal pollution usually occurs in a cyclical order, starting with industry and ending with people. Numerous pathways exist for heavy metals to infiltrate the dairy production system. These techniques include air deposition, applying inorganic fertilizers on land, using agrochemicals, animal manures, and animal feed. However, sewage irrigation may lead to a number of environmental issues, chief among them being heavy metal pollution of the soil. Raising agents, leaching from containers and inadvertent contamination during storage and marketing, and post-contamination throughout different production phases can all lead to higher amounts of heavy metals in milk and milk products (Abdeljalil *et al*., 2021). They can interfere with crucial metabolic processes, posing a serious risk to plant and animal health. In general, heavy metals interfere with metabolic process in two ways: In the beginning, they build up and impair the function of important organs and glands such the heart, brain, kidneys, bone, liver, etc. Second, they remove essential nutritional minerals from the body where they provide for biological activity (Abdeljalil *et al*., 2021).

Cadmium and lead are well known toxic metals, even when taken in small quantities. Children have been shown to be more sensitive to cadmium and lead than adults (Abdeljalil *et al*., 2021). Common air contaminants like lead and Cadmium are released into the atmosphere as a result of several industrial processes. Despite the low air levels, they do play a role in soil deposition and accumulation. These metals are contaminated by various industrial processes, resulting in soil, water, food, and plant contamination. This contamination puts humans and animals' health at risk by introducing these metals into the food chain (Asayehegn *et al*., 2021).

3. Materials and method

3.1 Study area

The study was conducted in Addis Ababa, the capital city of Ethiopia, which is in the country's central region. Milk drinks consumption is becoming common in Addis Ababa due to its easy accessibility and high preference by the potential consumers (children). Addis Ababa has 11 sub cities and all of the sub cities are considered for sample collection purpose.

3.2. Materials and chemicals

A market survey was carried out at supermarkets of the eleven sub cities of Addis Ababa to identify the commonly sold commercial milk drinks. Following the survey, different flavored (apple, orange, strawberry, mango) milk drinks were used for the current study. The samples were purchased from selected shops from each sub city of Addis Ababa. All chemicals used were of analytical grade.

3.3 Sample collection

Two milk drink brands were considered in this study. The two milk drink brands used in the experiment were brand A and brand B. From each brands, four milk drink samples from brand A and four milk drink samples from brand B with different flavor (apple, orange, strawberry and mango flavored) were collected from shops of each sub-city. Therefore, a total 88 samples (44 from each brand) were collected from the 11 sub cities. Amount of milk used for analysis was 6.6 litters for both brands. Shops were purposely selected to collect sample of both brands in one shop. Brands were identified and labeled with letters like brand A and brand B for the purpose of the study. Further labeling like S1, S2, S3 and S4 was used to differentiate the four flavored samples of each brand.

3.4. Study design

The study was conducted by CRD experimental research design method. The study was laboratory based experiment. The experiments were conducted at the Ethiopian public health institute (EPHI), Ethiopian food and drug authority (EFDA), Kolfe industrial college and Center for Food Science and Nutrition of Addis Ababa University.

3.5 Physicochemical analysis

3.5.1. Physical Analysis

3.5.1.1. Determination of pH

The pH of the milk drink was evaluated in the lab using a digital pH-meter (HANNA instrument, H1 9025 microcomputer). The pH of the milk drink sample was tested after the pH meter was calibrated using buffers with pH values of 7.0 and 4.0 each time.

3.5.1.2. Determination of specific gravity

Ultrasonic milk analyzer, milkotronic ltd model, Lacto Scan Company, Bulgaria city, supply 230 vac was used in the Kolfe industrial college to examine the Physical parameters of specific gravity of milk drinks.

The lactoscan was washed three times in distilled water after being cleansed with lactoscan cleaning mix 3% lactodaily and 97% distilled water. 25mL samples were taken in the sample tube and put in the sample holder one at a time with the analyzer in the recess position. Then when the starting button activated, the analyzer sucks the milk drink, takes the measurements, and returns the milk drink in the sample tube. Finally the specific gravity was shown on the digital indicator.

3.5.2. Chemical composition (protein, fat and solid not fat)

Ultrasonic milk analyzer, milkotronic ltd model, Lacto Scan Company, Bulgaria city, supply 230 vac was used in the Kolfe industrial college to examines the nutritional composition (protein, fat and solid not fat).

The lactoscan was washed three times in distilled water after being cleansed with lactoscan cleaning mix 3% lactodaily and 97% distilled water. 25mL samples were taken in the sample tube and put in the sample holder one at a time with the analyzer in the recess position. Then when the starting button activated, the analyzer sucks the milk drink, takes the measurements, and returns the milk drink in the sample tube. Chemical composition which includes solid fat, protein, fat and density was shown on the digital indicator as described in Asefa and Teshome (2019).

3. 6. Microbiological analysis

3.6.1. Sampling, sample processing and media preparation

Samples were collected and transported using cold chain and analyzed within 24 hours after collection. Dehydrated commercial microbiological media were prepared according to the manufacturer's instruction. Distilled water was used to reconstitute the media and sterilization was carried out by means of steam sterilization in an autoclave at 121^{o}C for 15 minutes. All glass wares such as petri-dishes, bottles, test tubes etc used for microbiological analysis were properly washed, dried and sterilized by means of dry heat sterilization in a hot air oven at 160 ^{o}C for 2 hrs.

3.6.2. Enumeration of total aerobic plat bacterial count

Enumeration of TPC in sample was done by pour plate technique according to standard procedures recommended by Nordic Committee on Food Analysis (NMKL), No 86, 2006 method**.** The media used for these micro-organisms was plate count agar and buffer peptone water solution diluents. Using separate sterile pipits, the 25mL of homogenous portion of milk drink sample was transferred to 225mL of prepared buffer peptone water in vortex the initial dilution solution was prepared and others appropriate and homogenate by transferring 1mL of the previous dilution to 9mL of diluents as a decimal dilution of 10^{-2} 10^{-3} and 10^{-4}. One mL of each dilution was pipette into two sterilized marked Petri dishes and added (20-25mL) plate count agar. After agar medium were mixed thoroughly and uniformly by different rotation and back-and-forth motion of plates on a flat level surface. Agar was solidified and solidified Petri dishes were inverted and incubated at 37 ^{o}C for 24 hours. A total of 30 to 300 colonies per plate were chosen, and colonies were counted as described in Abate *et al*. (2015).

3.6.3. Enumeration total coliform count (TCC)

The media used were; Violet red bile agar, Tryptone soya agar and Brilliant green broth and the diluents was peptone water. Twenty five ml of liquid milk sample was transferred to 225 ml of diluents to make 10^{1} dilutions. After shaking the first dilution well 1 ml of a sample was transferred to a test tube containing 9 ml of peptone water to make 10^{2} serial dilutions. From the 10^{2} dilution, 1 ml of the sample was transferred to a test tube containing 9 ml of the diluents to make 10^{3} serial dilutions. The preparation of the dilution factor was conducted until 10^{4} were obtained. One milliliter of each successive dilution was pipetted into each marked duplicate plate. Ten ml of the molten trypton soya agar kept at 45°c in to each Petri dish and the dilution

was mixed. After waiting for an hour 15 ml of the Violet red bile agar kept at 45°c in to each Petri dish. The dishes were incubated inverted at 37°c for 24 hours. For confirmation colonies were inoculated in to brilliant green bile broth and incubated at 37°c for 48 hours and production of gas and turbidity was observed (NMKL, 2004).

3.6.4. Enumeration *E. coli*

E. coli was determined according to the method of Nordic Committee on Food Analysis (NMKL), No 125, 2005.Twenty five ml of liquid milk sample was transferred to 225 ml of diluents to make 10^1 dilutions. After shaking the first dilution well 1 ml of a sample was transferred to a test tube containing 9 ml of peptone water to make 10^2 serial dilutions. The preparation of the dilution factor was conducted until 10^4 were obtained. One mL of each dilution was pipette into two sterilized marked Petri dishes and added 10 mL of tryptone soya agar after a hour violet red bile (15 mL) was added on top of the agar and incubated at 37°C for 24 hours after the incubation the typical colonies were inoculated using sterilized wire lope in to BGB broth test tube. From one plate 5 number of typical colony was taken and transferred to 5 BGB test tubes and incubated at 37 ^{0}C for 48 hours after the incubation production of gas and turbidity was observed. One wire loop of turbid brilliant green bile broth was inoculated in to Escherichia coli broth and incubated at 45 ^{0}C for 48h after the incubation of EC broth One wire loop of EC broth test tube was inoculated in to Nutrient broth and incubated at 45 ^{0}C for 24h finally indole was dropped in to nutrient broth test tube after 30 minutes the red, green and yellow ring was marked. The nutrient broth test tube saw Red ring color confirms the colonies as *E. coli*. Results are expressed as a percentile based on the number of positive samples.

3.6.5. Enumeration *Salmonella spp*

Salmonella pre-enrichment, selective enrichment, isolation and confirmation in homogenized milk drink samples was done by horizontal method for the detection of Salmonella species according the recommendation of NMKL, No 71, 2005 method. The 25 mL of homogenous portion of milk drink sample was transferred to 225 mL of prepared buffer peptone water and incubate at 37 ^{o}C for 24h. Using separate sterile pipets one mL of pre incubated samples were transferred in to 9mL of Rappaport vassilidis medium (RVM) broth and incubated at 42 ^{o}C for 24h after incubation production of gas and turbidity was observed. One wire loop of the turbid Rappaport vassilidis was streaked on the pre-dried surfaces of SS agar plate and incubated at 37 ^{o}C for 24h after the incubation appearance of small black-centered colonies was indicative of the

presence of Salmonella spp. Finally the black-centered colonies were biochemically examined for confirmation. Kligler Iron Agar, Lysine iron agar, Urease, Simmons Citrate Agar and indole test was conducted for the biochemical confirmation.

3.6.5.1 Biochemical Identification

Kligler Iron Agar

To detect the fermentation of glucose and lactose as well as the production of H2S, butt was stabbed and the slant was streaked and incubated at 35 ^{o}C for 24hrs. The presence of alkaline (red) slant and acid (yellow) butt, with or without production of H_2S was considered as positive Salmonella (Tafesse *et al*., 2014).

Lysine Iron Agar (LIA) (Oxoid)

The suspected organism was stabbed in to Lysine iron agar medium without disturbing the surrounding and incubated at 37 ^{o}C for 24hrs to assess oxidative deamination of lysine on the slant and decarboxylaton of lysine in the butt. The presence of alkaline (purple) reaction in both butt and slant of the tube was considered presumptive for Salmonella (Tafesse *et al*., 2014).

Urea Agar (Oxoid)

The suspected organism was stabbed in to urea agar medium without disturbing the surrounding and the tube was incubated at 37 ^{o}C for 24 hrs to assess the utilization of urea. The presence of no color change was considered as negative and thus presumptive for Salmonella (Tafesse *et al.*, 2014).

Simon's Citrate Agar (Oxoid)

The agar was prepared according to the manufactures instruction. Suspected salmonella colony was streaked on slant surface and stub on the butt Agar and incubated at 37 ^{o}C for 24h to investigate utilization of citrate. The presence of growth and color change from green to blue was considered as presumptive for Salmonella. A negative reaction is indicated by inhibition to poor growth without change in color (Tafesse *et al*., 2014).

Indole test /Kovac's reagent/

Suspected salmonella colony was inoculated in to Nutrient broth and incubated 37^{0}C for 24h after the incubation of nutrient broth indole was dropped in to nutrient broth test tube after 30 minutes the red, green and yellow ring was marked. Presence of Red color ring considered as indole positive test and thus not for presumptive Salmonella spp.

3.5.6. Enumeration *Staphylococcus aureus*

Staphylococcus aureus was determined according to the method of NMKL, No 125, 2005**.** The media used for these micro-organisms was Mannitol salt agar base (MSA) and peptone salt solution diluents. Twenty five(25ml) of a sample was transferred to glass bottles containing 225 ml of buffer peptone water to make 10^{-1} dilution and mix and homogenize the content using shaker. For inoculation 0.1mL of the sample of the initial suspension 10^{-1}dilution using duplicate plates spread onto solidifies MSA plates and incubated at 37 ^{o}C for 24 hours. Yellow Golden colonies were counted as suspected *S. aureus* for confirmation of *S. aureus,* typical colony was chosen from MSA plates and sub cultured into Tryptic soy agar (TSA) plate, and incubated at 37 ^{o}C for 24 hours. The test was confirmed by coagulate test. Appearance of coagulation (clot) in the test tube after incubation for 24 hours at 37^{o}C was indicative of the presence of *Staphylococcus aureus.*

3.6.7. Enumeration Yeast and mould

Yeast and mould were counted according to NMKL, No 98, 1997 using Rose Bengal-chloramphenicol agar spread method and incubated for 5-7 days at 25°C.

For all testes of microbial count of milk drinks colony was calculated by the following formula.

$\sum C / (1\times n1+0.1\times n2)\, d$

Where: $\sum C$ = Sum of all colonies on all plates counted

n1 = Number of plates in the first dilution counted

n2 = Number of plates in the second dilution counted

d = Dilution factor of the lowest dilution used

3.7. Heavy metal Analysis

3.7.1. Cleaning Apparatus

Prior to heavy metal (Chromium, Cadmium and Lead) analysis, all glassware were washed with distilled water, soaked in nitric acid (69%) for 24 hours, then rinsed with distilled water and dried over oven. To prevent contamination, the glassware was kept in a clean environment as described in Alemu (2008).

3.7.2. Digestion of milk drink samples for metal determination

The samples were digested by the wet digestion technique (LODU, 2017) to prepare a clear colorless sample solution that is suitable for the analysis. 2 mL of milk drink was measured for each of flavored milk drinks and brands, and it was transferred to a 50 mL falcon's tube for digestion. The milk drink sample was mixed with 7 mL of 69% concentrated HNO_3 and 2 mL of 60% $HClO_4$. Each batch of digestion sets included the preparation of analytical blanks. The sample and blank was swirled gently to homogenize the mixture. Then, the digestion falcon's tube containing the sample and blank was digested on water bath at 90ºC until clear and color less solution was obtained. After heating, the digestion vessels were opened and the sample was allowed to cool to room temperature to prevent foaming and splashing. To remove any suspended or turbid residues, the cold clear solution was filtered through Whitman filter paper (0.45µm pore diameter membrane) into a 50 mL falcon tube. Distill water was then added to dilute the solution to the desired level. Following filtration, all of the digested samples were kept refrigerated until the Microwave Plasma Atomic Emission Spectrometer (MP-AES) was used to measure the concentrations of each metal in the sample solutions.

3.7.3. Standard preparation

The concentration measurements for lead, cadmium and chromium were determined from a working curve after calibrating the instrument with standard concentrations. To find out how much trace metal was present in the solutions from the milk drink sample, calibration curves were drawn. To do this, intermediate solutions was prepared for cadmium, lead and chromium. Which were prepared by taking 2.5 mL for each metal transfer into 50 mL volume from the stock standard solutions containing 1000 mg/L, 2% HNO_3 was added to a level of 50 mL. Using 10 mL and 25 mL of volume, a series of working standard solutions were prepared using the formula $v_1c_1 = v_2c_2$ from intermediate standard solutions of the corresponding metals. After the instrument is calibrated properly, each sample's metal concentration was determined. Six-point calibration curves were established by introducing the prepared working standard solutions in MP-AES. Plotting the working standard concentration (mg/L) versus the corresponding intensity of each metal allowed for the determination of the calibration curves' correlation coefficient (R^2).

Table 4. Concentration of standard solution used to calibrate the instrument and their corresponding correlation coefficients for the determination of heavy metals

Heavy	Metals	Conc. of	Conc. of	Correlation	Regression equation

metals	Conc. of stock solutions (mg/L-)	intermediate solution (mg/L)	standard series (mg/L)	coefficient(R^2)	
Chromium	1000	50	0.1, 0.2, 0.4, 0.8, 1.6,3.2	0.9998	Y= 32846x +71.226
Cadmium	1000	50	0.1, 0.2, 0.4, 0.8, 1.6,3.2	0.9994	Y= 13796x + 529.4
Lead	1000	50	0.1, 0.2, 0.4, 0.8, 1.6,3.2	0.9999	Y= 3143.3x – 19.6

3.7.4. Instrumentation / Instrumental analysis

Agilent Technologies 4210 MP-AES was used for heavy metal analysis for cadmium (Cd), Lead (Pb), and Chromium (Cr). MP-AES Wavelength, temperature program and other instrumental parameters was adjusted according to the guidelines provided in the instrument manual.

The instrument and metal parameters are listed in Table 5. The MP-AES analyzed the samples in triplicates, and intensity values were converted into concentration values against the metal calibration curves using Agilent MP-expert software.

Table 5. MP-AES 4210 operational conditions for metal determination in milk drink

Elements	Wavelength (nm)	Nebulizer Flow (L/min)	Replicates	Pump Speed (rpm)	Sample Uptake Time (s)	Stabilization time (s)	Read Time (s)
Cr	425.433	0.9	3	15	30	15	3
Cd	228.802	o.5	3	15	30	15	3
Pb	405.781	0.75	3	15	30	15	3

3. 8. Percentage of the main raw materials

The main raw materials used for the preparation of milk drinks are water and milk. To determine the minimum amount of milk to be used for the preparation of milk drinks, at least some of the nutrients for which milk is known have to be there in the milk drinks. For this purpose, the study used the codex guideline for nutrition and health claims (Codex CAC/CL 23) and codex

guideline on nutrition labeling (Codex CAC/GL 2). The first guideline was used to obtain the conditions needed for milk drinks to be considered as a source for specific nutrients (in our case protein, calcium and vitamin B12). This guideline uses nutrient reference value (NRV) of nutrients for calculating any food to be considered as a source for a specific nutrient. Therefore, the second guideline (Codex CAC/GL 2) was used to obtain NRVs of the nutrients (protein, calcium and vitamin B12). After obtaining the NRVs, the conditions specified in the first guideline (Codex CAC/CL 23) was considered to determine the minimum amount of milk to be used for making milk drinks which could be considered as a source for protein, calcium and vitamin B12.

Table 6.Nutrient composition of whole cow milk and the two codex guide line used to calculate percentage of raw material (milk and water)

Nutrients	Nutrient composition of whole cow milk[a]	NRV[b]	Contribution to NRV to be claimed source for the specific nutrient (%)[c]
Protein	3.06(g)	50	5
Calcium	122 (mg)	800	7.5
Vitamin B12	0.45 (ug)	1	7.5

NRV = Nutrient reference value
a: obtained from EPHI food composition table
b: obtained from codex guideline on nutrition labeling (Codex CAC/GL 2)
c: obtained from codex guideline for nutrition and health claims (Codex CAc/CL 23)

3. 9 Statistical method

The statistical analyses of the data were performed using one-way analysis of variance (ANOVA), with SPSS version 20 and Microsoft office excel. All the data were analyzed in triplicates except for microbial analysis which was analyzed in duplicate. Results were expressed as the mean ± standard deviations. To ascertain the significance differences among means of samples, Duncan's multiple range tests was used at p value less than 0.05.

3.10 Ethical clearance

Ethical approval was obtained from institutional review board of College of Natural and Computational Sciences of Addis Ababa University.

4. Results and discussion

4.1 physical properties of milk drinks

4.1.1 pH

In general, the pH levels of milk drinks obtained from markets in Addis Ababa varied between 3.92 ± 0.04 and 4.24 ± 0.03. Notably, there was a statistically significant difference in pH between brand A and brand B, as illustrated in Table 7. Brand A exhibited a pH range of 4.18 ± 0.03 to 4.24 ± 0.03, with an average pH value of 4.22 ± 0.05. In contrast, the pH of brand B ranged from 3.92 ± 0.04 to 3.96 ± 0.07, and the average pH value was 3.95 ± 0.05. Examining various flavors, our investigation revealed that there were not significant differences in pH values attributable to flavor variations for both brands ($p > 0.05$).

Our findings indicate lower pH values compared to the results reported by Asefa and Teshome (2019), where the pH of milk collected from Debrezeit and Sebeta milk sheds was noted to be 6.02 and 6.17. This divergence might be attributed to the inclusion of different acids such as citric acid and lactic acid during the preparation of milk drinks, as explicitly mentioned on the labels of the milk drink bottles. Another potential factor could be improper storage conditions for the milk beverages, leading to acid development through lactose fermentation and subsequent reduction in the pH of the milk (Asefa and Teshome, 2019).

4.1.2 Specific gravity

As shown in the table below (table 7), the experimental results of specific gravity of milk drinks obtained from markets in Addis Ababa varied between 1.012 ± 0.13 and 1.032 ± 0.28 at 20 C^o.

Specific gravity of brand A milk drinks ranged from 1.031 ± 0.29 to 1.032 ± 0.28, with an average specific gravity value of (1.032 ± 0.46 g/ mL). In contrast, the specific gravity of brand B ranged from 1.012 ± 0.13 to 1.012 ± 0.13, and the average specific gravity value was (1.012 ± 0.29 g/ mL). However, the specific gravity milk drinks there was not significant differences in specific gravity values attributable to flavor variations for both brands ($p > 0.05$).

Our findings indicate that brand B exhibits specific gravity values lower to those reported by Bruktawit and Ashenafi, (2016), ranging from 1.027 g/ mL-1.035 g/ mL at 16C^o. However, brand B shows similar specific gravity levels compared to their reported values. This divergence might be attributed to the inclusion of addition of more water during the preparation of milk drinks. Addition of more water was leading to subsequent reduction in the milk specific gravity of milk drinks (Bruktawit and Ashenafi, 2016).

4.1.3 Comparison of physical properties of milk drinks with the Ethiopian whole cow milk standard

Ethiopian milk drink standard does not provide specific limits for physical properties but physical property was compared with Ethiopia whole cow milk.

The average pH value of both brand A and brand B milk drinks (4.22±0.05 and3.95±0.05) respectively did not fulfilled the Ethiopia whole cow milk which specifies a minimum pH level of milk 6.60-6.80.

Similarly the comparison of experimental specific gravity result with the Ethiopia whole cow milk standard. Average brand A specific gravity levels of milk drinks (1.032±0.86 g/ mL) obtained from markets in Addis Ababa were above the minimum limit of specific gravity specified in Ethiopia whole cow milk standard (1.026 g/mL– 1.032 g/mL at 20 C^o). In contrast, the average specific gravity value of brand B milk drinks (1.012±0.05 g/ mL) not fulfilled the specific gravity requirement specified in the Ethiopia whole cow milk standard.

Table 7 Comparison of physical property of two milk drinks with Ethiopia whole cow milk standard

Brand	Flavors	pH		Specific gravity (g/ mL)	
		Experimental result	ES limit for cow's milk	Experimental result	ES limit for cow's milk at 20 C^o
A	Mango	4.24±0.03[b]	6.60-6.80	1.031±0.29[b]	1.026– 1.032
	Orange	4.23±0.04[b]		1.032±0.28[b]	
	Strawberry	4.21±0.09[b]		1.032±0.87[b]	
	Apple	4.18±0.038[b]		1.031±0.39[b]	
	Average	4.22±0.05[b]		1.032±0.86[b]	
B	Mango	3.95±0.03[a]		1.013±0.13[a]	
	Orange	3.95±0.06[a]		1.012±0.36[a]	
	Strawberry	3.96±0.07[a]		1.012±0.56[a]	
	Apple	3.92±0.04[a]		1.012±0.13[a]	
	Average	3.95±0.05[a]		1.012±0.36[a]	

Results are expressed as Mean ± SD. Means followed by different superscript letters along the column are significantly different (P<0.05).

4.2 Chemical composition of milk drinks

The result of chemical composition of milk drinks sample indicated in Table 8. Important factors that show the nutritional value of milk drinks are their chemical composition. In this study protein, fat and solid non-fat was considered as an important chemical composition as it is specified in the Ethiopian milk drink standard.

4.2.1 Protein

In general, the protein levels of milk drinks obtained from markets in Addis Ababa ranged between 1.27 ± 0.03 and 3.15 ± 0.08. Notably, there was a statistically significant difference in protein between brand A and brand B, as illustrated in Table 8. Brand A exhibited a protein range of 3.03 ± 0.04 to 3.20 ± 0.14, with a significantly higher average protein value of 3.12 ± 0.10. In contrast, the protein of brand B ranged from 1.27 ± 0.03 to 1.3 ± 0.08, and the average protein value was 1.28 ± 0.07. Examining various flavors, our investigation revealed that there were not significant differences in protein values attributable to flavor variations for Brand A ($p > 0.05$).

Our findings reveal that brand A exhibits protein values similar to those reported by Asefa and Teshome (2019), Keba (2020), and Bruktawit and Ashenafi (2016) for pasteurized milk, ranging from 3.08% to 3.28%, 3.01%, and 3.2%, respectively. However, brand B shows lower protein levels compared to their reported values. In contrast, both brands in our study demonstrate higher protein content than the results documented by Nnubia et al. (2020), who found protein levels in milk drinks commercially available in Nigeria ranging from 0.75 ± 0.16 to 1.31 ± 0.11. This discrepancy suggests that variations in the formulations of milk drinks among brands contribute to a substantial degree of variability in the nutrient composition of these beverages, even when sharing the same flavor base.

4.2.2 Fat

As shown in the table below (table 8), the experimental results of fat of milk drinks obtained from markets in Addis Ababa varied between 0.43 ± 0.09% and 1.19 ± 0.07%. Notably, there was a statistically significant difference in fat between brand A and brand B, as illustrated in Table 8. Brand A exhibited a fat range of 0.43 ± 0.09 to 0.57 ± 0.06, with significantly lower average fat value (0.50 ± 0.11%) as compared to brand B. In contrast, the fat of brand B ranged from 1.09 ± 0.11to 1.19 ± 0.07, and the average fat value was 1.13 ± 0.12. Examining various

flavors, our investigation revealed that there were not significant differences in fat values attributable to flavor variations for both brands ($p > 0.05$).

Our results indicate decreased values when compared to the findings reported by Keba (2020), who noted a fat content of 3.63% in milk collected from the Oromia region. Furthermore, there is a lower fat content in all milk drinks collected from various sub-cities in comparison to the results documented by Nnubia et al. (2020), where the fat content of commercially sold milk drinks in Nigeria ranged from 1.29 ± 0.07 to 2.37 ± 0.04%. This disparity suggests that the observed difference may be attributed to the addition of more water instead of milk during the preparation of milk drinks by vendors. Another potential factor contributing to the lower fat values could be the removal of cream, as discussed by Adam (2009).

4.2.3 Solid not Fat

All solid not fat (SNF) levels of milk drinks obtained from markets in Addis Ababa varied between 3.47 ± 0.07% and 8.57 ± 0.23%. Notably, there was a statistically significant difference in SNF between brand A and brand B, as illustrated in Table 8. Brand A exhibited a SNF range of 8.24 ± 0.09 to 8.57 ± 0.23, with an average SNF value of 8.43 ± 0.19. In contrast, the SNF of brand B ranged from 3.47 ± 0.07 to 3.58 ± 0.24, and the average SNF value was 3.47± 0.19. It clearly indicating that brand A milk drinks have significantly higher SNF than brand B milk drinks. After examining various flavors, our investigation revealed that there were no statistically significant differences in SNF values linked to flavor variations for both brands ($p > 0.05$).

Our results reveal lower SNF values in brand B and comparable SNF values in brand A when compared to the reported findings by Keba (2020) and Bruktawit and Ashenafi (2016), where the SNF content of milk was documented as 8.20% and 7.6%, respectively. This discrepancy may be attributed to the addition of cheap bulking ingredients, like cheap flour, and the removal of milk fat as cream, both of which raise total solids (Shehzadi and Khan, 2016). Additionally, other potential factors influencing the nutrient composition of milk drinks include the genetics and breed of the animal, environmental conditions, stage of lactation, parity, and the nutrition of the cow (Jenkins and McGuire, 2006).

4.2.4 Comparison of chemical composition of milk drinks with the Ethiopian milk drink standard

We compared the examined chemical composition, including protein content, fat content, and solids-not-fat, with the Ethiopian milk drink standard. The average Fat value of brand A milk drinks (0.5 ± 0.11%) did not fulfilled the Ethiopian milk drink standard which specifies a

minimum fat level of 1.1%. In contrast, the average fat value of brand B milk drinks (1.13 ± 0.12%) fulfilled the fat requirement specified in the Ethiopian milk drink standard.

Regarding the comparison of experimental protein result with the ES value, all protein levels of milk drinks obtained from markets in Addis Ababa were above the minimum limit of protein specified in Ethiopian milk drink standard (1%). Both brand A and brand B milk drinks fulfilled the ES requirement with an average protein level of 3.12 ± 0.10 and 1.28 ± 0.06 %, respectively.

Similarly, comparison of experimental SNF content result with the ES specification/limit revealed that all SNF levels of milk drinks obtained from markets in Addis Ababa were above the minimum SNF level of the Ethiopian milk drink standard (2.9%). Both brand A and brand B milk drinks fulfilled the SNF requirements specified in ES with an average SNF values of 8.43 ± 0.19 and 3.47 ± 0.19%, respectively.

Table 8.Comparison of chemical composition of milk drink with Ethiopia milk drink standard

Brand	Flavors	Protein (%)		Fat (%)		SNF (%)	
		Experimental result	ES Minimum limit	Experimental result	ES Minimum limit	Experimental result	ES Minimum limit
A	Mango	3.03±0.04^{b}		0.57±0.06^{a}		8.24±0.09^{b}	
	Orange	3.20±0.14^{b}		0.53 ± 0.09^{a}		8.50±0.15^{b}	
	Strawbery	3.15±0.08b		0.47 ± 0.13^{a}		8.57±0.23^{b}	
	Apple	3.08 ±0.04^{b}		0.43 ± 0.09^{a}		8.38±0.09^{b}	
	Average	3.12±0. 10^{b}		0.5 ± 0.11^{a}		8.43±0.19^{b}	
B	Mango	1.3 ±0.08^{a}		1.09 ±0.11^{b}		3.36±0.17^{a}	
	Orange	1.29±0.07^{a}		1.16 ±0.14^{b}		3.58±0.24^{a}	
	Strawbery	1.27 ±0.07^{a}		1.10 ± 0.13^{b}		3.48±0.18^{a}	
	Apple	1.27±0.03^{a}		1.19 ± 0.07^{b}		3.47±0.07^{a}	
	Average	1.28±0.06^{a}	1	1.13±0.12^{b}	1.1	3.47±0.19^{a}	2.9

Results are expressed as Mean ± SD. Means followed by different superscript letters along the column are significantly different (P<0.05). SNF= Solid Not-Fat; ES = Ethiopian standard

4.3 Microbial quality

Table 9 displays the total microbial count, Coli forms and yeast, mould, along with the presence or absence of *Salmonella* and *S. aureus* and *E.coli* that were obtained from the assessment of milk drink brands.

4.3.1 Total plate count

The amount of bacteria in a sample that can grow and form countable colonies on plate count agar after being incubated at 37 °C for 24 hours is known as the total viable bacterial count. In relation to the degrees of overall microbial contamination, total plate counts are used as a measure of microbiological quality.

The total plate counts levels of milk drinks obtained from markets in Addis Ababa varied between 0.8 log 10 CFU/ mL to 2.64 log 10 CFU/ mL as indicated in table 9. Brand A's milk drinks displayed an elevated range of total plate counts, spanning from 0.93 log 10 CFU/ mL to 2.64 log 10 CFU/ mL , and an average total plate count value of 1.48 log 10 CFU/ mL . Conversely, brand B's total plate counts were lower, ranging from 0.8 log 10 CFU/ mL to 2.17 log10 CFU/ mL, with an average total plate count value of 1.31 log 10 CFU/ mL.

Our total plate count findings revealed values that were lower than those reported by Ntuli et al (2016), where the total plate counts of milk ranged from 2.2 to 4.8 log 10 CFU/ mL. This variance could be linked to potential cross-contamination during the preparation of milk drinks and milk collection. Another contributing factor might be inadequate storage conditions for the milk beverages, subsequently leading to an increase in the total plate counts of the milk (Koushki et al., 2016). The total microbial count serves as an indicator of the hygienic quality of food products.

4.3.2 Total Coliform count

The TCC levels of milk drinks obtained from markets in Addis Ababa varied between 0 log10 CFU/ mL to 1.02 log 10 CFU/ mL as illustrated in Table 9. Brand A milk drinks exhibited higher total coliform counts range of 0 log 10 CFU/ mL to 1.02 log CFU/mL, with an average total coliform count value of 0.5 log 10 CFU/ mL. In contrast, the total coliform count of brand B milk drinks had lower ranged from 0 log10 CFU/mL to 0.49 log10 CFU/ mL and the average total coliform count value was 0.33 log 10 CFU/ mL.

Our coliform count results indicated lower total coliform count values compared to the results reported by Ntuli *et al*, (2016) and Mikru *et al*, (2021), where the total coliform count of milk

was noted to be on the range between 0 to 2.5 log 10 CFU/ mL. This divergence might be attributed to either defect in pasteurization process or post pasteurization contamination which includes contamination in packaging materials, defects in pipe lines (Jamal *et al*., 2018), subsequently increases the total coliform counts of the milk drink As we know that presence of coliform bacteria indicate fecal contamination making the quality of the industry not satisfactory.

4.3.3 *Escherichia coli*

In the current study, the *E.coli percent* levels of milk drinks obtained from markets in Addis Ababa varied 0% to 9.09% as illustrated in Table 9. Brand A and brand B milk drinks exhibited similar *E.coli percent* range of 0% to 9.09% with an average *E.coli* value of 4.55%.

Our *E.coli* results indicated lower *E.coli* values compared to the results reported by weldemedhin, (2018) where they reported that *E.coli* on the pasteurized milk was 6.2% and our result was almost near to samples analyzed in South Africa done by Koushki *et al*, (2016) where they reported that *E. coli* percent on the pasteurized milk was 3.9%. The difference could be due to contamination with waste water and fecal materials (Vahedi *et al*, 2013).

4.3.4 S. aureus, Salmonella spp, Yeast and mold

***S. aureus, Salmonella spp,* Yeast and mold*:* -** were not detected in all of the studied samples (for both brand A and brand B) regardless of the flavors used for making the milk drinks as shown in table 9 this study was in a clear agreement with the research output done by Aberra, (2010) where they reported that *salmonella* on pasteurized milk was not isolated.

4.3.5 Comparison of microbial quality of two milk drink brands with Ethiopia milk drink standard

The analyzed microbial count was compared with the specific microbial requirement of Ethiopian standard for the milk drink specification. As shown in the table below (table 9), the analyzed microbial count was compared with the Ethiopian milk drink standard microbial requirement.

Regarding the comparison of experimental Total plate count result with the ES value, all Total plate count levels of milk drinks obtained from markets in Addis Ababa were above the minimum limit of Total plate count specified in Ethiopian milk drink standard (1 log 10 CFU/ mL). Both brand A and brand B milk drinks not fulfilled the ES requirement with an average Total plate count level of 1.48 log 10 CFU/ mL and 1.31 log 10 CFU/ mL, respectively. This is good indicator for monitoring the sanitary conditions practiced during handling of milk,

collection, production, pasteurization deficiency, secondary contamination, and type of packaging.

Similarly, comparison of experimental Total coliform count result with the ES specification/limit revealed that all Total coliform count levels of milk drinks obtained from markets in Addis Ababa were above the minimum Total coliform count level of the Ethiopian milk drink standard (Nil). Both brand A and brand B milk drinks not fulfilled the TCC requirements specified in ES with an average TCC values of 0.5 log 10 CFU/ mL and 0.33 log 10 CFU/ mL, respectively.

Looking at, comparison of experimental *E.coli* result with the ES specification/limit revealed that all *E.coli* value of brand A and brand B milk drinks did not fulfilled the Ethiopian milk drink standard which specifies a minimum *E.coli* limit of (nil). Both brand A and brand B milk drinks not fulfilled the *E.coli* requirements specified in ES with an average (4.55%) for both brands.

Similarly comparison of analyzed *S.aureus salmonella* and yeast and mould with the ES value both brand A and brand B produced milk drinks had below the ES value (0, -ve and 0) respectively. All *S.aureus salmonella* and yeast and mould levels of milk drinks obtained from markets in Addis Ababa results were belowe the Ethiopia milk drink standard value. Ethiopian milk drink standard recommended that milk drink *S.aureus salmonella spp* and yeast and mould shall comply with the requirement of nil for both *S.aureus* and *salmonella spp and* 1 log CFU/ mL for yeast and mould.

Table 9.Comparison of microbial quality of two milk drink brands with Ethiopia milk drink standard

Brand type	Flavors	parameter tested (Log10 CFU/mL)								Absent and present		percentile	
		TPC/ mL	ES Max. Limit	TCC mL	ES Max. limit	Yeast and mould	ES Max. limit	*S.aureu*	ES Max.limit	*Salmonell spp*	ES Max. limit	*E coli*	ES Max. limit
A	Mango	1.1	1	0.49		0		0		-ve		9.09	
	Orange	1.3		0.51		0		0		-ve		0	
	Strawbery	2.64		1.02		0		0		-ve		9.09	
	Apple	0.9		0		0		0		-ve		0	
Average		1.48		0.5	Nil	0	1	0	Nill		Nil	4.55	Nil

	Mango	0.8		0		0		0		-ve		9.09
	Orange	1.3		0.49		0		0		-ve		0
	Strawbery	2.17		0.41		0		0		-ve		0
B	Apple	0.9		0.42		0		0		-ve		9.09
Average		1.31		0.33		0		0		-ve		4.55
Total average		1.4		0.42		0		0		Ve		4.55

TPC: total plate count; TCC: total coliform count; CFU: colony forming unit

4.4 Heavy metals

Table 10 shows that the three metals were present in all varieties and brands of milk drinks. It is well known that a variety of factors, such as feeding practices, the use of different water sources, and industrial pollution, can cause a large variation in the amount of heavy metals in milk (Asayehegn *et al*., 2021).

4.4.1 Chromium

In Addis Ababa's markets, the chromium (Cr) levels in milk drinks generally ranged from 0.04 ± 0.02 to 0.18 ± 0.05 mg/L. Notably, a statistically significant difference in Cr was observed between brand A and brand B, as indicated in Table 10. Brand A showed a Cr range of 0.15 ± 0.15 to 0.18 ± 0.05, with an average Cr value of 0.17 ± 0.08. In contrast, brand B's milk drinks had Cr levels ranging from 0.04 ± 0.02 to 0.14 ± 0.02, with an average Cr value of 0.09 ± 0.03. These results clearly demonstrate that, on average, brand A's milk drinks had a significantly higher Cr value (0.17 ± 0.08 mg/L) compared to the average Cr value of brand B's milk drinks (0.09 ± 0.03 mg/L). When considering various flavors, our investigation found no significant differences in Cr values due to flavor variations for both brand A and B ($p > 0.05$).

Our findings align with the research conducted by Alem et al (2015), wherein they reported that the chromium (Cr) concentration in milk closely resembled our results, with a mean value ranging from 0.055 to 0.075 mg/L. Our results indicated higher Cr values compared to the findings reported by Belete et al (2014), Akele et al (2017), and Asayehegn et al (2021), where the Cr levels in milk were noted to be 0.845 to 0.895 mg/L, 0.468 to 0.828 mg/L, and 0.00 mg/L to 0.4 mg/L, respectively. This disparity might be attributed to potential contamination, which could occur incidentally during the use of potable water for cleaning equipment on the farm or in the processing plant (Wang et al, 2016) and during the preparation of milk drinks

4.4.2 Lead

In Addis Ababa's markets, the Lead (Pb) levels in milk drinks generally ranged from 0.38 ± 0.09 to 0.60 ± 0.10 mg/L. Notably, a statistically significant difference in Pb was observed between brand A and brand B, as indicated in Table 10. Brand A showed a Pb range of 0.38 ± 0.09 to 0.48 ± 0.23, with an average Pb value of (0.45± 0.15 mg/L). In contrast, brand B's milk drinks had Pb levels ranging from 0.50 ± 0.16 to 0.60 ± 0.10, with an average Pb value of (0.56 ± 0.12 mg/L). These results clearly demonstrate that, on average, brand B's milk drinks had a significantly higher Pb value (0.56 ± 0.12mg/L) compared to the average Pb value of brand A's milk drinks (0.45± 0.15mg/L). When considering various flavors, our investigation found no significant differences in Pb values due to flavor variations for both brand A and B ($p > 0.05$).

our findings indicate lower Pb values compared to the finding report by Abdulkhaliq *et al* (2012) in Palestine, where the Pb of milk was noted to be 0.0 to 0.39 mg/L and higher research output done by Ahmad *et al* (2016) in Bangladesh, where they reported that the Pb concentration of the milk were noted to be 0 – 0.93 mg/L. This divergence might be attributed to the inclusion of tap water during the preparation of milk drinks because; many heavy metals were reported in drinking water (Chowdhury *et al*, 2016). Consumption of contaminated animal feed and commonly used drinking water might be another contributing factor.

4.4.3 Cadmium

In Addis Ababa's markets, the cadmium (Cd) levels in milk drinks generally ranged from 0.94 ± 0.43 to 1.25 ± 0.76 mg/L. Notably, a statistically significant difference in Cd was not observed between brand A and brand B, as indicated in Table 10. Brand A showed a Cd range of 1.08 ± 0.69 to 1.25 ± 0.76, with an average Cd value of 1.16 ± 0.73. In contrast, brand B's milk drinks had Cd levels ranging from 0.94 ± 0.43 to 1.17 ± 0.85, with an average Cd value of 1.02 ± 0.39. These results clearly demonstrate that, on average, brand A's milk drinks had a significantly higher Cd value (1.16 ± 0.73 mg/L) compared to the average Cd value of brand B's milk drinks (1.02 ± 0.39mg/L). When considering various flavors, our investigation found no significant differences in Cd values due to flavor variations for both brand A and B ($p > 0.05$).

our findings indicate lower Cd values compared to the finding reported by Malhat *et al*, (2012) in Egypt, Abou-Arab *et al*, (2008) in Egypt, Abdulkhaliq *et al*, (2012)) in Palestine, where the Cd of milk was noted to be 0.20 to 0.29 mg/L , 0.016 to 0.04 mg/L, 0.022 to 0.057 mg/L respectively. Food is the primary cause of cadmium exposure. There are numerous harmful impacts of this metal on human health (Pastorelli *et al*., 2023). This is a useful indicator for

monitoring on the hygienic practices used during the manufacturing and collection of milk, as well as secondary contamination and packaging type.

4. 4.4 Comparison of heavy metals of milk drink with Ethiopia milk drink standard

The analyzed heavy metals concentration was compared with the specific heavy metals concentration limits of Ethiopian standard for the milk drink specification. As shown in the table below (table 10), the analyzed heavy metals concentration was compared with the Ethiopian milk drink specification standard heavy metals concentration.

Looking at, comparison of experimental Lead content result with the ES specification/limit revealed that all Lead levels of milk drinks obtained from markets in Addis Ababa were above the minimum Lead level of the Ethiopian milk drink standard (0.02mg/L). Both brand A and brand B milk drinks fulfilled the Lead requirements specified in ES with an average Lead values of 0.45 ± 0.15 and 0.56 ± 0.12mg/L, respectively.

Lead has no advantageous biological role and is known to accumulate in the body. Because lead (Pb) is a neurotoxic that permanently interrupts normal brain development, exposure to Pb can have negative health effects, particularly in young children and pregnant women (Asayehegn *et al*., 2021).

Similarly, Comparison of experimental cadmium content result with the ES specification/limit revealed that all cadmium levels of milk drinks obtained from markets in Addis Ababa were above the minimum cadmium level of the Ethiopian milk drink standard (0.05mg/L). Both brand A and brand B milk drinks not fulfilled the cadmium requirements specified in ES with an average cadmium values of 1.16 ± 0.73 and 1.02 ± 0.39 mg/L, respectively.

Regarding Chromium, it was not included in Ethiopian milk drink standard to know more about the product Cr was studied. The comparison of experimental Chromium result with the Ethiopian whole cow milk standard value, all Chromium levels of milk drinks obtained from markets in Addis Ababa were below the minimum limit of Chromium specified in Ethiopian whole cow milk standard (0.3). Both brand A and brand B milk drinks fulfilled the ES requirement with an average Cr level of 0.17 ± 0.08 and 0.09 ± 0.03 mg/L respectively. Cr is essential to maintain the metabolic systems of human body. However, it can result in more serious poisoning that can cause convulsions, kidney and liver damage, upset stomach and ulcers, and even death (Asayehegn *et al*., 2021).

Table 10.Comparison of Heavy metals (Cr, Pb and Cd) status of two brands of milk drinks with Ethiopia milk drink standard with four flavors

Brand type	Flavors	Cr (mg/L)	ES maximum limit	Pb (mg/L)	ES maximum limit	Cd (mg/L)	ES maximum limit
	Mango	0.16±0.08[b]	0.3	0.46 ± 0.12[a]	0.02	1.22±0.93[a]	
	Orange	0.18± 0.08[b]		0.48 ±0.23[a]		1.08±0.69[a]	
	Strawberry	0.15±0 .15[b]		0.47 ±0.15[a]		1.13±0.84[a]	
	Apple	0.18±0 .05[b]		0.38± 0.09[a]		1.25±0.76[a]	
A	Average	0.17±0.08[b]		0.45± 0.15[a]		1.16± 0.73[a]	
	Mango	0.05± 0.03[a]		0.50 ± 0.16[b]		1.17±0.85[a]	
	Orange	0.13±0 .03[a]		0.60 ± 0.10[b]		0.94±0.43[a]	
	Strawberry	0.04±0 .02[a]		0.55 ± 0.13[b]		1.11±0.75[a]	
	Apple	0.14± 0.02[a]		0.57 ± 0.10[b]		1.11± 0.72[a]	
B	Average	0.09± 0.03[a]		0.56±0.12[b]		1.02±0.39[a]	0.05

Results are expressed as Mean ± SD. Means followed by different superscript letters along the column are significantly different (P<0.05).

4.5. Percentage of main raw materials (milk and water) of milk drinks

As it is clearly depicted in table 11, for the milk drink to be claimed as a source of protein, the calculated amount of whole milk to be used was found to be 81.69% with 18.31% water. Similarly for the milk drink to be claimed as a source of calcium, 49.18% whole milk is required to be mixed with 50.82% water and to be claimed as a source of vitamin B12, 16.67% whole milk was found to be mixed with 83.33% water.

Milk is considered as one of the nutritious food and important for human health, specifically to children. However, its nutritional value could be decreased by the addition of extra water. Consuming milk of poor quality could be harmful to people's health (Asged and El Zubeir, 2021). As per the calculation made using Codex CAc/CL 23 and Codex CAC/GL 2, milk drink should constitute at least 81.69% of whole milk and the remaining around 19% could be water so as to be considered as a source for all of the three nutrients considered in this study (protein, calcium and vitamin B12) as indicated in table 11 below.

Table 11.Calculated percentages of main raw materials (milk and water) of milk drinks to make it protein, calcium and vitamin B12 source

Nutrients	Calculated amount of whole milk to be added (%)	Calculated amount of water to be added (%)
Protein	81.69	18.31
Calcium	49.18	50.82
Vitamin B12	16.67	83.33

5. Conclusions and Recommendations

5.1 Conclusions

Based on the study's findings, it is possible to draw the following conclusions about milk drinks collected from Addis Ababa city market. Physical properties such as pH in both brands and specific gravity in brand A milk drinks does not fulfill Ethiopian cow milk standard. The chemical composition of fat's in brand B as well as microbiological quality that includes TPC, TCC, and E. Coli in both brands does not complained Ethiopian milk drink standard and also heavy metals Pb and Cd does not complained the requirement set by ES for milk drink. Milk drink percentage of raw materials shows higher percentage of cow milk to be added to make minimum protein amount.

5.2 Recommendations

The study's conclusion led to the following suggestions

- Milk drink specification to be revised considering physical properties requirements
- It is important to regularly inspection of milk drinks that are marketed, to make sure they meet to the minimal requirements set by law.
- It's important to keep the general hygiene standards surrounding to the handling and production of milk drinks.
- Milk drink factory must be producing the product by using these two codex guide lines

6. References

Abate, M., Wolde, T., & Nigussie, A. (2015). Bacteriological quality and safety of raw cow's milk in and around Jigjiga City of Somali Region, Eastern Ethiopia. *Int J Res Stud Biosci*, 3, 48-55.

Abbasi, S., & Mohammadi, S. (2013). Stabilization of milk–orange juice mixture using Persian gum: Efficiency and mechanism. *Food Bioscience*, 2, 53-60.

Abdeljalil, N. A., Benkhayal, A. A., Ahmida, M. H. S., El Sharaa, I. A., & Ahmida, N. H. (2021). Determination of Lead and Cadmium in Different Types of Milk Samples Collected from Different Markets in Benghazi-Libya

Abdulkhaliq A, Swaileh K, Hussein M & Matani M (2012). Levels of metals (Cd, Pb, Cu and Fe) in cow's milk, dairy products and hen's eggs from the West Bank, Palestine. *International Food Research Journal*, 19: 1089-1094.

Abebe, B., Zelalem, Y., & Ajebu, N. (2013). Handling, processing and utilization of milk and milk products in Ezha district of the Gurage zone, Southern Ethiopia. *Journal of Agricultural Biotechnology and Sustainable Development*, 5(6), 91-98

Aberra, A. M. (2010). Microbiological safety of pasteurized and raw milk from milk processing plants in and around Addis Ababa (Doctoral dissertation, Addis Ababa University).

Abou-Arab A, Abou-Donia M & Enb A (2008). Chemical composition, metals content and pesticide residues in raw, pasteurized and UHT milk and their dietary intake. *Journal of the Egyptian Society of Parasitology*, 39: 111-121.

Ahmad M, Roy S, Sarwar N, Morshed S, Alam M, Matin A & Kobra T (2016). Contamination of raw fresh milk, market pasteurized milk and powdered milk by toxic heavy metals in Bangladesh. *Scientific Research Journal*, 4: 19-24.

Ahmed, M. A., Ehui, S., & Assefa, Y. (2004). Dairy development in Ethiopia. Intl Food Policy Res Inst.

Akbay, C., & Tiryaki, G. Y. (2008). Unpacked and packed fluid milk consumption patterns and preferences in Turkey. *Agricultural Economics*, *38*(1), 9-20.

Akele M, Abebe D, Alemu A, Assefa G & Madhusudhan A (2017). Analysis of trace metal concentrations in raw cow's milk from three dairy farms in North Gondar, Ethiopia: chemometric approach. *Environment Monit Assess*, 3: 499-511.

Alem G, Tesfahun K & Kassa B (2015). Quantitative determination of the level of selected heavy metals in the cows' milk from the Dairy Farm of the Haramaya University, Eastern Ethiopia. *International Journal of Chemical and Natural Science*, 3: 240-248.

Alemu, A. (2008). Levels of Essential and Toxic Metals in Commercial Powdered Infant Formulas (Doctoral dissertation, Addis Ababa University).

Asayehegn, A. T., Hymete, A., Hagos, S., & Ashenef, A. (2021). Determination of the Level of Heavy Metals in Cow's Milk Collected from Butajirra and Meskan Districts, South Central Ethiopia.

Asefa, Z., & Teshome, 2019. Physical properties and chemical compositions of raw cow milk in milk shades around Addis Ababa, Ethiopia. *Journal of Natural Sciences Research*, 9(19), 33-37.

Asged, A. A., & El Zubeir, I. E. M. (2021) Occurrence of adulterants and preservatives in the Milk Sold in Rural areas of Omdurman, Sudan

Ayza, A., & Yilma, Z. (2014). Patterns of milk and milk products adulteration in Boditti town and its surrounding, South Ethiopia. *J Agric Sci*, 4(10), 512-6.

Belete T, Hussen A & Rao M (2014). Determination of concentrations of selected heavy metals in cow's milk: Borena Zone, Ethiopia. *Journal of Health Science*, 4: 105-112.

Bolarinwa, I. F., Aruna, T. E., Adejuyitan, J. A., Akintayo, O. A., & Lawal, O. K. (2018). Development and quality evaluation of soy-walnut milk drinks. *International Food Research Journal*, 25(5), 2033-2041

Bruktawit, S., & Ashenafi, M. (2016). Physicochemical properties and microbial quality of cow milk collected from selected subcity of Addis Ababa, Ethiopia. Addis Ababa University, College of veterinary Medicine and Agriculture, Department of Animal Production Studies

Caswell, H. (2009). The role of fruit juice in the diet: an overview. Nutrition Bulletin, 34(3), 273-288

Chowdhury, S., Mazumder, M. J., Al-Attas, O., & Husain, T. (2016). Heavy metals in drinking water: occurrences, implications, and future needs in developing countries. *Science of the total Environment*, *569*, 476-488.

Coburn, B., Grassl, G. A., & Finlay, B. B. (2007). Salmonella, the host and disease: a brief review. *Immunology and cell biology*, 85(2), 112-118

Coburn, B., Grassl, G. A., & Finlay, B. B. (2007). Salmonella, the host and disease: a brief review. *Immunology and cell biology*, 85(2), 112-118

d'Aoust, J. Y., Emmons, D. B., McKellar, R., Timbers, G. E., Todd, E. C. D., Sewell, A. M., & Warburton, D. W. (1987). Thermal inactivation of Salmonella species in fluid milk. *Journal of Food Protectio*n, 50(6), 494-501

Desye, B., Bitew, B. D., Amare, D. E., Birhan, T. A., Getaneh, A., & Gufue, Z. H. (2023). Quality assessment of raw and pasteurized milk in Gondar city, Northwest Ethiopia: A laboratory-based cross-sectional study. *Heliyon*, *9*(3).

Dey, S., & Karim, M. H. (2013). Study on physicochemical and microbial quality of available raw, pasteurized and UHT milk during preservation. *International Journal of Science Inventions Today*, 2(2), 150-7

Dirar, H. A. (1993). The indigenous fermented foods of the Sudan: a study in African food and nutrition. *CAB international*

ESA 2021 Ethiopian milk drink specification Second edition ICS: 67.100.10 Published by Ethiopian Standards Agency © ESA milk drink Specification

FAO. (1990). (Food and Agriculture Organization). The Technology of Traditional Milk Products in Developing Countries. *Animal Production and Health Paper*. No. 85. Food and Agriculture Organization of the United Nations. Rome, Italy

Fusco, V., Chieffi, D., Fanelli, F., Logrieco, A. F., Cho, G. S., Kabisch, J., ... & Franz, C. M. (2020). Microbial quality and safety of milk and milk products in the 21st century. *Comprehensive Reviews in Food Science and Food Safety*, 19(4), 2013-2049.

Getabalew, M., Alemneh, T., & Akeberegn, D. (2019). Dairy production in Ethiopia-existing scenario and constraints. *Biomed J Sci Tech Res*, 16, 12304-9.

Getaneh, D. (2022). Assessments of Raw Cow Milk Quality on Some Selected Districts of Ayer Tena, Ghion And Koble in Bahir Dar City (Doctoral dissertation).

Górska-Warsewicz, H., Rejman, K., Laskowski, W., & Czeczotko, M. (2019). Milk and dairy products and their nutritional contribution to the average polish diet. *Nutrients*, 11(8), 1771

Guetouache, M., Guessas, B., & Medjekal, S. (2014). Composition and nutritional value of raw milk. *J Issues Biol Sci Pharm Res*, 2350, 1588

Gutema. A. K .(2022) . Review On Current Milk Production Status, Existing Production Constraints and Opportunities in Ethiopia. *concept of dairy veterinary*, 5(1).

Gutierrez, E., Metcalfe, J. J., & Prescott, M. P. (2022). The Relationship between Fluid Milk, Water, and 100% Juice and Health Outcomes among Children and Adolescents. *Nutrients*, 14(9), 1892

Hahn, G.(1996).Pathogenic bacteria in raw milk-Situation and significance. Symposium on bacteriological quality of raw milk, Wolfpassing, Austria.PP.60-64.

Hingne, P., & Chavan, S. D. (2021). Effect of incorporation of herbs on physico-chemical properties of flavoured milk. *Journal of Pharmacognosy and Phytochemistry*, 10(2), 270-275

Hoddinott, J., Headey, D., & Dereje, M. (2015). Cows, missing milk markets, and nutrition in rural Ethiopia. *The Journal of Development Studies*, 51(8), 958-975.

Hossin, M. S., Debnath, S., Alam, M. N., Islam, M. S., Miah, M. K., Mollah, M. S. H., & Saeid, A. (2021). Physico-chemical, sensory and microbiological characteristics of strawberry flavored milk under refrigerated storage. *Asian Journal of Dairy and Food Research*, 40(1), 82-87

Islamoğlu, F., Torul, O., Yazici, E., Duran, C., & Selvi, E. K. (2020). Determination of Some Metal Contents of Cow's Milk in Akçabaat District of Trabzon through the ICP-MS Method. *Turkish Journal of Analytical Chemistry*, *2*(2), 62-68.

Jakobsen, M., & Narvhus, J. (1996). Yeasts and their possible beneficial and negative effects on the quality of dairy products. *International dairy journal*, *6*(8-9), 755-768

Jamal, J. B., Akter, S., & Uddin, M. A. (2018). Microbiological quality determination of pasteurized, UHT and flavoured milk sold in Dhaka, Bangladesh. *Stamford Journal of Microbiology*, *8*(1), 1-6.

Jay,J.M.,Loessner,M.J.,&Golden,D.A.(2008).Modern food microbiology. *Springer science & Business Media*. Seventh edition

Jenkins, T. C., & McGuire, M. A. (2006). Major advances in nutrition: impact on milk composition. *Journal of dairy science*, *89*(4), 1302-1310. .

Kailasapathy, K. (2015). Chemical composition, physical, and functional properties of milk and milk ingredients. *Dairy processing and quality assurance*, 77-105.

Keba Wirtu, A. (2020). Assessment of Dairy Food Environment, Physicochemical Properties, and Microbial Safety of Milk and Cottage Cheese across the Value Chain in Oromia Region, Ethiopia (Doctoral dissertation, Addis Ababa University).

Keba, A., Rolon, M. L., Tamene, A., Dessie, K., Vipham, J., Kovac, J., & Zewdu, A. (2020). Review of the prevalence of foodborne pathogens in milk and dairy products in Ethiopia. *International dairy journal*, 109, 104762

Kirdat, R. A., Patil, B. D., & Ramteke, S. P. (2019). Preparation of flavored milk using beetroot juice as natural colorant. *The Pharma Innovation Journal*, 8(12), 196-9.

Kopf-Bolanz, K. A., Schwander, F., Gijs, M., Vergères, G., Portmann, R., & Egger, L. (2012). Validation of an in vitro digestive system for studying macronutrient decomposition in humans. *The Journal of Nutrition*, 142(2), 245-250.

Koushki, M., Koohy-Kamaly, P., Azizkhani, M., & Hadinia, N. (2016). Microbiological quality of pasteurized milk on expiration date in Tehran, Iran. *Journal of dairy science*, *99*(3), 1796-1801

Lemma, H. D., Mengistu, A., Kuma, T., & Kuma, B. (2017). The potential of milk production and consumption for improving nutrition of smallholder dairy households in Ethiopia. *Milk Science International-Milchwissenschaft*, 70(3), 10-16

Lewis, J. 2019. Codex nutrient reference values. Rome. FAO and WHO.

Lodu, c. (2017). determination of selected heavy metals in vegetables and soil along the shore lines of lake hawassa and tikur wuha river (doctoral dissertation).

Mahato, D. K., Keast, R., Liem, D. G., Russell, C. G., Cicerale, S., & Gamlath, S. (2020). Sugar reduction in dairy food: an overview with flavoured milk as an example. *Foods*, 9(10), 1400

Malhat F, Hagag M, Saber A & Fayz E (2012). Contamination of cows milk by heavy metal in Egypt. *Bulletin of Environmental Contamination and Toxicology*, 88: 611-613.

Merwan, A., Nezif, A., & Metekia, T. (2018). Review on milk and milk product safety, quality assurance and control. *International Journal of Livestock Production*, 9(4), 67-78.

Mikru, A., Adane, M., & Dobo, B. (2021). Microbial hazard analysis in the pasteurized milk production value chain at a commercial dairy plant in Hawassa, southern Ethiopia. *Journal of Advances in Dairy Research*, *9*, 254

Milk adulteration by adding water and starch at Khartoum state. *Pakistan Journal of Nutrition*, *8*(4), 439-440.

Mohammed I, Zahra M & Zakari A (2017). Determination of some heavy metals in milk of cow grazing at selected areas, of Kaduna Metropolis. *International Journals of Advanced Research in Computer Science and Software Engineering*, 7: 341-346.

Mudgil, D., & Barak, S. (2019). Dairy-based functional beverages. *In Milk-based beverages* (pp. 67-93). Woodhead Publishing

Nnubia, U. I., Ezeonyeche, C. L., Nnodim, E. J., Okechukwu, F. O., & Joseph, B. C. June 2020 .Effect of sunlight exposure on the nutrient composition of two selected commercial milk drinks. *Journal of Dietitians Association of Nigeria (JDAN)* Volume 11 Number 1, 2635-3326

Ntuli, V., Njage, P. M. K., & Buys, E. M. (2016). Characterization of Escherichia coli and other Enterobacteriaceae in producer-distributor bulk milk. *Journal of dairy science*, *99*(12), 9534-9549.

Ortuzar, J., Martinez, B., Bianchini, A., Stratton, J., Rupnow, J., & Wang, B. (2018). Quantifying changes in spore-forming bacteria contamination along the milk production chain from farm to packaged pasteurized milk using systematic review and meta-analysis. *Food Control*, 86, 319-331

Pastorelli, G., Michele, S., Marisanna, S., & Everaldo, A. (2023). Milk quality determined using chemical analysis and microwave plasma atomic emission spectrometry as a function of seasonality in two conventional Italian dairy farms. Acta Scientiarum Polonorum. *Technologia Alimentaria*, *22*(1), 5-12.

Pelletier, D. L., Frongillo Jr, E. A., Schroeder, D. G., & Habicht, J. P. (1995). The effects of malnutrition on child mortality in developing countries. *Bulletin of the World Health Organization*, 73(4), 443.

Ray, B., & Bhunia,A. 2013).fundamental food microbiolog fifth edition

Reta, M. A., & Addis, A. H. (2015). Microbiological quality assessment of raw and pasteurized milk. *International Journal of Food Science and Microbiology*, 2(6), 087-091

Saha, S., & Ara, A. (2012). Chemical and microbiological evaluation of pasteurized milk available in Sylhet city of Bangladesh. *The Agriculturists*, 10(2), 104-108

Sebastian, R. S., Goldman, J. D., Enns, C. W., & LaComb, R. P. (2010). Fluid milk consumption in the United States. What We Eat In America, *NHANES* 2005-2006

Shehzadi, R., Yu-Qing, T., & Khan, M. I. (2016) Composition and Adulteration Analysis of Milk Samples from Ten Different Towns of Lahore

Sherbon, J. W. (1988). Physical properties of milk. *In Fundamentals of dairy chemistry* (pp. 409-460). Springer, Boston, MA.

Shi, C., & Knøchel, S. (2021). Sensitivity of Molds From Spoiled Dairy Products Towards Bioprotective Lactic Acid Bacteria Cultures. *Frontiers in microbiology*, *12*, 631730.

Soliman, G. Z. (2005). Comparison of chemical and mineral content of milk from human, cow, buffalo, camel and goat in Egypt. *The Egyptian Journal of Hospital Medicine*, 21(1),

Sousa, A., & Kopf, K. A. (2017). Nutritional Implications of an Increasing Consumption of Non-Dairy Plant-Based Beverages Instead of Cow's Milk in Switzerland. *Advances in Dairy Research*, 5(04), 1-7.

Tabassum, S., & Uddin, M. A. (2016). Microbiological analysis of different flavoured milk samples collected from various areas of Dhaka city, Bangladesh. Bangladesh *Journal of Microbiology*, 33(1-2), 11-15.

Tafesse, F., Desse, G., Bacha, K., & Alemayehu, H. (2014). Microbiological quality and safety of street vended raw meat in Jijiga town of Somali Regional State, southeast Ethiopia. *Microbiology research*

Teklu, A., Hymete, A., Gebreyesus, S. H., & Ashenef, A. (2023). Determination of the level of heavy metals and assessment of their safety in cow's milk collected from Butajira and Meskan districts, south central Ethiopia. *Environmental Monitoring and Assessment*, *195*(1), 206.

Tesfay, T., Kebede, A., & Seifu, E. (2013). Quality and safety of cow milk produced and marketed in Dire Dawa Town, Eastern Ethiopia. *Int J Integr Sci Innov Technol*, *2*, 1-5.

Vahedi, M., Nasrolahei, M., Sharif, M., & Mirabi, A. M. (2013). Bacteriological study of raw and unexpired pasteurized cow's milk collected at the dairy farms and super markets in Sari city in 2011. *Journal of preventive medicine and hygiene*, *54*(2), 120.

Wang, A., Duncan, S. E., Knowlton, K. F., Ray, W. K., & Dietrich, A. M. (2016). Milk protein composition and stability changes affected by iron in water sources. *Journal of Dairy Science*, *99*(6), 4206-4219.

Weldemedhin, M. S., & Kindie, M. S. (2018). Addis Ababa university college of health school of allied department of medical.

Workicho, A., Belachew, T., Feyissa, G. T., Wondafrash, B., Lachat, C., Verstraeten, R., & Kolsteren, P. (2016). Household dietary diversity and animal source food consumption in Ethiopia: evidence from the 2011 welfare monitoring survey. *BMC public health*, 16(1), 1-11.

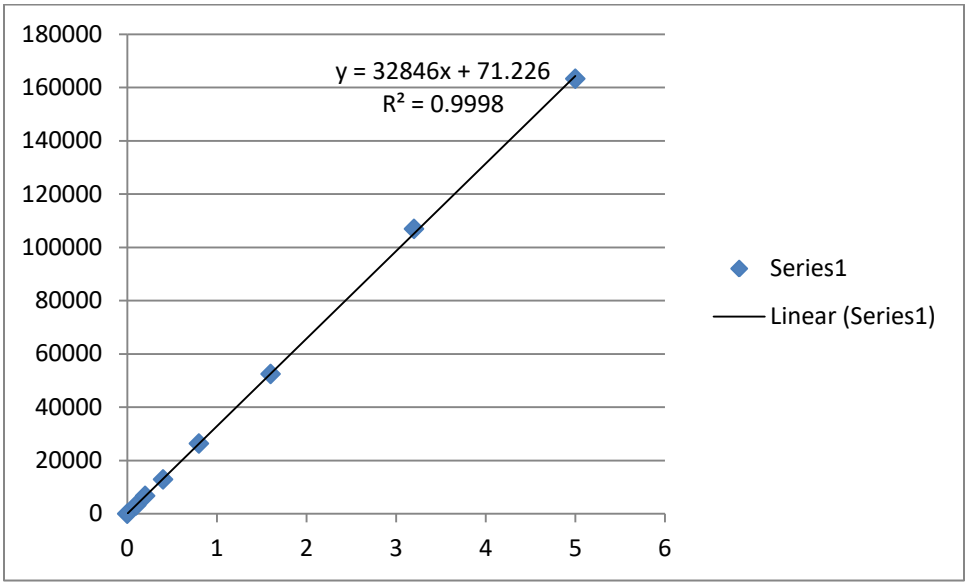
Yilma, Z., Guernebleich, E., Sebsibe, A., & Fombad, R. (2011). A review of the Ethiopian dairy sector. Ed. Rudolf Fombad, Food and Agriculture Organization of the United Nations, Sub Regional Office for Eastern Africa (FAO/SFE), Addis Ababa, Ethiopia, 81

Zebib, H., & Zewdu, A. (2020). Nutritional Quality, Level of Chemical Contaminants and Adulterants in Milk and Dairy Products in Ethiopia: A Review. *Modern Chemistry*, 8(4), 64

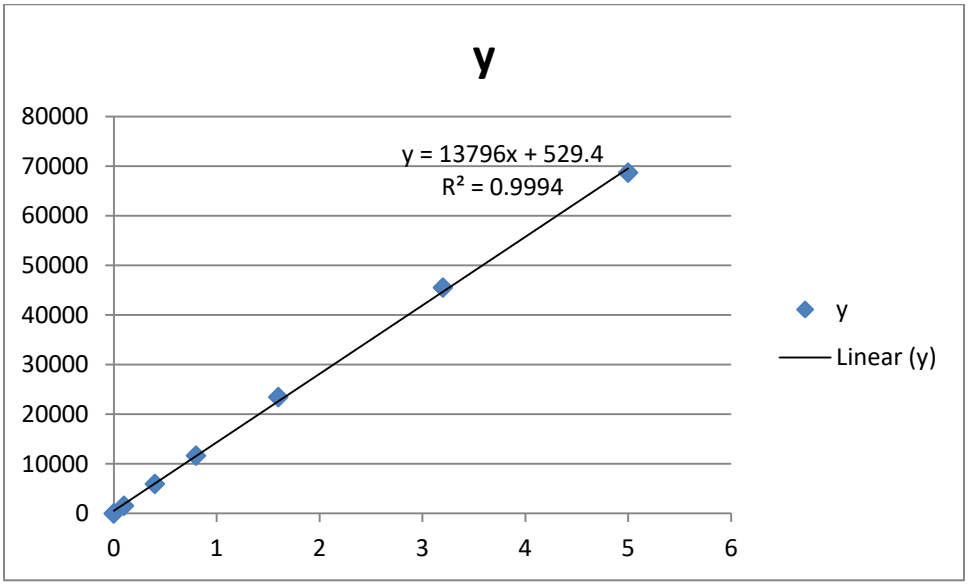
Zhang, L., Wei, Q., Han, Q., Chen, Q., Tai, W., Zhang, J., & Xia, X. (2018). Detection of Shigella in milk and clinical samples by magnetic immunocaptured-loop-mediated isothermal amplification assay. *Frontiers in microbiology*, *9*, 94.

7. ANNEXIES

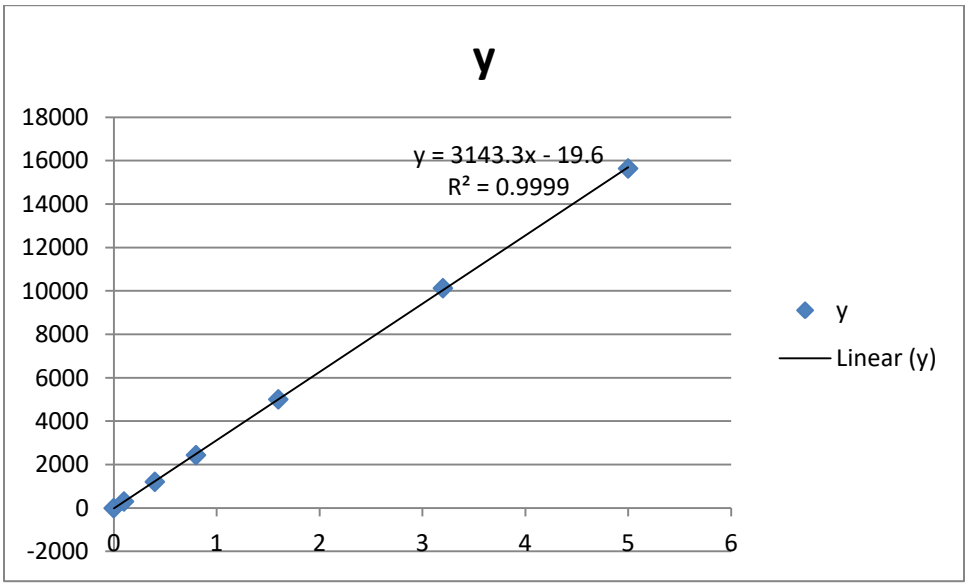
Appendix 1: Standard Calibration curve of Chromium



Appendix 2: Standard Calibration curve of Cadmium



Appendix 3: Standard Calibration curve of Lead



Appendix 4: Manuscript

Article

Evaluation of Physicochemical properties, microbial quality, and percentage of raw materials and heavy metals of milk drinks in Addis Ababa

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Abstract

Milk and milk products are nutrient rich foods. But, it becomes a health risk to the consumers if not handled properly due to high perishability and vulnerability to microorganisms. The present study is primarily aimed to evaluate physicochemical properties and microbial quality of milk drinks sold in Addis Ababa city and to determine percentage of raw materials (milk and water) to make it a protein, calcium and vitamin B12 source. A cross sectional study was conducted in all sub city of Addis Ababa and purposive sampling was conducted to collect two milk drink brands from each sub city. A total of 88 milk drink samples were assessed in the study. The study was analyzed by NMKL method, MP-AES instrument, Lacto scan milkotronic ltd instrument and using Codex Guide lines. Physical parameters pH, density, chemical composition protein, fat ,SNF ,microbial quality TPC,TCC,E. coli, S.aures, salmonella spp, yeast and mould, heavy metals Cr, Pb and Cd were tasted in these study. All of the physicochemical and heavy metals of the milk drinks was significantly different in milk drink brands at ($p<0.05$) and were not significantly different in milk drink flavor variation at ($p>0.05$) except Cd. significantly highest pH and density was observed in brand A milk drinks (4.22±0.05 and 31.62±0.86) respectively. The mean value of highest fat content was observed in brand B milk drinks (1.13±0.12%) and the lower in brand A milk drinks (0.50 ± 0.11%) and also the highest TPC and TCC was on brand A milk drinks (1.48 log 10 CFU/ mL and 0.548 log 10 CFU/ mL) respectively and lowest in brand B milk drinks (1.31 log 10 CFU/ mL and 0.33 log 10 CFU/ mL) respectively. While E.coli should not be detected in heat treated foods, E.coli was detected in 4 out of 88 (4.55%) samples. None of the samples found to be positive for S. aureus, salmonella spp, Yeast and mould. The highest Cr and Cd contamination was observed in brand A milk drinks (0.17±0.08 and 1.16± 0.73 mg/L) respectively and lowest in brand B milk drinks (0.09± 0.03 and 1.02±0.39 mg/L) respectively. The highest Pb contamination was observed in brand B milk drinks (0.56±0.12 mg/L) and lowest in brand A milk drinks (0.45± 0.15 mg/L). All pH and density of the milk drinks had not fulfilled the Ethiopian whole cow milk requirement except brand A density of milk drinks. Similarly chemical compositions of the milk drinks had fulfilled the Ethiopian milk drink Standards except

brand A fat milk drink. The microbial quality of TPC, TCC and E.coli and also heavy metals, Pb and Cd of milk drinks was not complain Ethiopian milk drink Standards. For the milk drink to be claimed as a source of protein, Ca & vitamin B12, it should be made with a minimum of 82 % whole milk add remaining could be water. Generally speaking, the physicochemical, microbial quality and heavy metals of milk drinks was found to be poor. Therefore, to ensure safety and quality of milk drinks, it is suggested to follow scientific justification behind the formulation of milk drinks and there should be a hygienic practice on milk drink production and handling.

Keywords: Milk drink; Microbial quality; Raw materials

1. INTRODUCTION

1.1. Background

Drinks are typically made from a range of plant and animal-based ingredients, such as fruits, vegetables, cereal grains, and milk from mammalian glands (Bolarinwa *et al*., 2018). Drinks can increase nutrient intake and hydration (Gutierrez *et al*., 2022). Milk based drinks are the most acceptable food items for consumers in the functional food market (Hossin *et al*., 2021).

Milk is a very nutrient-dense food that provides both the macro and micronutrients required for human growth, development, and health maintenance (Zebib and Zewdu, 2020). It is primarily an excellent source of protein, fat, carbohydrates, vitamins, and minerals (Ayza and Yilma, 2014) and calcium, magnesium, sodium, potassium, and inorganic phosphate, citrate, are among the minerals. Milk contains several different kinds of vitamins. Vitamins A, D, B12, and riboflavin are common (Tabassum and Uddin, 2016).

Ethiopia has 10 million dairy cows, and they produce 3.2 billion liters of milk per year, or 1.54 liters per cow, each day, for a total of 180 days of lactation (Getabalew *et al.,* 2019). Low milk production results from a lack of feed availability and other factors related to the rainfall pattern (Getabalew *et al.,* 2019).

Many people in low and middle-income countries suffer from micro nutrient deficiencies. An important factors contributing to these deficiencies is the conception of mainly plant- based diet that are low in micro nutrients (Lemma *et al.,* 2017). In terms of the importance of milk and dairy products, changes in food choice determinants and consumption patterns (Górska-Warsewicz *et al.,* 2019). To provide certain nutrition and to meet the linkage between demand and supply of milk, milk drinks are a simple alternative to those who only drink plain water.

Milk drink is a drink obtained by mixing fermented or plain milk with potable water, along with or without other nondairy ingredients and flavorings (Nnubia *et al*., 2020).This is especially true

for the poor and those in developing country with a limited variety of dietary options. Commercial milk drinks are those that are made for consumers as ready-to-drink beverages (Nnubia *et al*., 2020).

Currently, healthy eating is encouraged as a method of preventing deficiencies diseases brought on by inadequate nutritional consumption (Nnubia *et al*., 2020). Consumers desire food that is healthy, wholesome, and nutrient-dense, as well as food that has been produced and prepared in a safe, sanitary manner and is pathogen-free. Quality dairy products are those that are without of dangerous poisonous chemicals and pathogenic microbes (Ayza and Yilma, 2014).

Milk's chemical composition creates a positive atmosphere for the pathogens to survive, grow and reproduce even though they are pasteurized or refrigerated (Jamal *et al*., 2018). Bacteria have the ability to produce heat-stable proteases and lipases, which are still active after pasteurization and can contaminate milk when kept for an extended period of time. After pasteurization, milk can also become contaminated (Fusco *et al*., 2020).

Milk and its products are the most consumed food by humans, especially children so it is important to investigate the extent of the toxic heavy metals and determine their concentrations in milk samples (Abdeljalil *et al*., 2021). At high concentrations, heavy metals are harmful due to their interference with normal metabolic process (Abdeljalil *et al*., 2021). Heavy metal pollution in milk and dairies can be caused by raw materials from milking due to the contamination of milking animals or by machinery and equipment in contact with dairies during production and storage (Islamoğlu *et al*., 2020).

As far as our knowledge in the area is concerned, there is no or little studies on milk drinks that could be due to the fact that milk drink is a relatively recent product, especially to Ethiopia. Therefore, this study aims at investigating the physicochemical properties and microbial quality of milk drinks sold in Addis Ababa city, Ethiopia. The study also focused on determining the percentage of the main raw materials (water and milk) of the milk drinks to make it as a source for some of the nutrients (protein, calcium, and vitamin B12).

2 MATERIALS AND METHODS

2.1. Materials and chemicals

A market survey was carried out at supermarkets of the eleven sub cities of Addis Ababa to identify the commonly sold commercial milk drinks. All chemicals used were of analytical grade (Sigma-Aldrich). The purchased samples were transported to Ethiopian public health institute laboratory and subjected to physicochemical and microbial quality analysis.

2.2. Sampling and sample preparation

Two milk drink brands were considered in this study. The two types of milk drinks used in the experiment were brand A and brand B. From each brands, four milk drink samples each with different flavor (apple, orange, strawberry and mango flavored) were collected from shops of each sub-city. Then, 8 milk drink samples were purchased for the two brands from each sub-city. Therefore, a total 88 samples (44 from each brand) were collected from the 11 sub cities. Amount of milk was used for analysis 6.6 litter for brand A and 4.4 litters for Brand B. The different volume was due to the size of the bottles, brand A contained 150 mL and brand B contained 100 mL. Shops were purposely selected to collect sample of both brands in one shop. Brands were identified and labeled with letters like brand A and brand B for the purpose of the study. Further labeling like S1, S2, S3 and S4 was used to differentiate the four flavored samples of each brand. A water-resistant marker was used for the labeling purpose.

2.3 Determination of pH

The pH of the milk drink was evaluated in the lab using a digital ph-meter (HANNA instrument, H1 9025 microcomputer). The pH of the milk drink sample was tested after the pH meter was calibrated using buffers with pH values of 7.0 and 4.0 each time.

2.4 Determination of physic chemical properties of milk drink

Lacto scan milkotronic ltd, supply 230 vac was used in the Kolfe industrial college to examine the Physical parameters of specific density, protein, Fat and SNF of milk drinks

2.5. Microbiological analysis

2.5.1. Sample processing and Media preparation

Samples were collected and transported using cold chain and analyzed within 24 hours after collection. Dehydrated commercial microbiological media were prepared according to the manufacturer's instruction. Distilled water was used to reconstitute and sterilization was carried out by means of steam sterilization in an autoclave at 121^{o}C for 15 minutes. All glass wares such as petri-dishes, bottles, test tubes etc used for microbiological analysis were properly washed, dried and sterilized by means of dry heat sterilization in a hot air oven at 160 ^{o}C for 2 hrs. To process the sample, 25g of milk drink was mixed with 225 ml of buffered peptone water. Further decimal serial dilutions were performed up to 10^4 as required in the same peptone water for bacteriological and mycological sample analyses.

2.5.2. Enumeration of total aerobic plat bacterial count

The milk drink samples were analyzed using plate count agar by incubating for 37°C at 24 hours. The bacteria were enumerated as Colony-Forming Units per mL (CFU/mL) .

2.5.3. Enumeration of total Coliform count

Coliform test was conducted using the Nordic Committee on Food Analysis, NMKL Method No. 44. About 5 ml of tryptone soya agar was poured to 1 ml of 1:10 to $1:10^4$ diluted samples and After waiting for an hour, 15 ml of VRBA was added. Plate count agar plates were inverted to incubate at 37 ^{o}C for 48 hours. Five colonies from presumptive coliforms were confirmed by checking for the production of gas in brilliant green bile salt lactose broth. Escherichia coli in EC broth was inoculated and incubated at 45 ^{0}C for 48h. The presence of gas indicated the occurrence of coliforms. E. coli was confirmed by indole test.

2.5.4. Identification of *Salmonella spp*

Salmonella spp was tested by NMKL, No 71, 2005. Using buffered peptone water pre-enrichment medium followed by Rappaport vascildas broths selective enrichments and salmonella and shigella agar (SS) Agar isolation medium incubated for 24 hours at 37°C. Salmonella presumptive colony was subculture on TSA plates and biochemically examined for confirmation. Kligler Iron Agar, Lysine iron agar, Urease, Simmons Citrate Agar and indole test was conducted for the biochemical confirmation.

***2.5.5.* Staphylococcus aureus**

Staphylococcus aureus was determined according to the method of NMKL, No 125, 2005. Using spread on to plate method on Mannitol salt agar base and incubated for 37 ^{o}C for 24 hours. The test was confirmed by coagulase test

2.5.6. Yeasts and Moulds Enumeration

Yeast and mould were counted according to NMKL, No 98, 1997 using Dichloran- Bengalrot-chloramphenicol (DRBC) spread method and incubated for 5 days at 25°C.

2.6. Heavy metal Analysis

All glassware were washed with distilled water, soaked in nitric acid (69%) for 24 hours, then rinsed with distilled water and dried over oven. The samples were digested by the wet digestion technique (LODU, 2017) to prepare a clear colorless sample solution that is suitable for the analysis. 2 mL of milk drink was measured for each of flavored milk drinks and brands, and it was transferred to a 50 ml falcon's tube for digestion. The milk drink sample was mixed with 7 mL of 69% concentrated HNO_3 and 2 mL of 60% HClO4. Each batch of digestion sets included the preparation of analytical blanks. Intermediate solutions were prepared for cadmium lead and

chromium. Which were prepared by taking 2.5 mL for each metal transfer into 50 ml volume from the stock standard solutions containing 1000 mg/L, 2% HNO_3 was added to a level of 50 ml. Series of working standard solutions were prepared by 10 ml and 25 ml of volume from intermediate standard solutions of the respective metals. Six point calibration curves were established by introducing the prepared working standard solutions in MP-AES.

Table 1 Concentration of standard solution used to calibrate the instrument and their corresponding correlation coefficients for the determination of heavy metals metal

Heavy metals	Metals Conc. of stock solutions (mgL-1)	Conc. of intermediate solution (mgL-1)	Conc. of standard series (mgL-1)	Correlation coefficient(R^2)	Regration equation
Chromium	1000	50	0.1, 0.2, 0.4, 0.8, 1.6,3.2	0.9998	Y= 32846x +71.226
Cadmium	1000	50	0.1, 0.2, 0.4, 0.8, 1.6,3.2	0.9994	Y= 13796x + 529.4
Lead	1000	50	0.1, 0.2, 0.4, 0.8, 1.6,3.2	0.9999	Y= 3143.3x – 19.6

2. 7. Percentage of the main raw materials

The main raw materials used for the preparation of milk drinks are water and milk. To determine the minimum amount of milk to be used for the preparation of milk drinks, at least some of the nutrients for which milk is known have to be there in the milk drinks. For this purpose, the study used the codex guideline for nutrition and health claims (Codex CAc/CL 23) and codex guideline on nutrition labeling (Codex CAC/GL 2). The first guideline was used to obtain the conditions needed for milk drinks to be considered as a source for specific nutrients (in our case protein, calcium and vitamin B12). This guideline uses NRV of nutrients for calculating any food to be considered as a source for a specific nutrient. Therefore, the second guideline (Codex CAC/GL 2) was used to obtain NRVs of the nutrients (protein, calcium and vitamin B12). After obtaining

the NRVs, the conditions specified in the first guideline (Codex CAc/CL 23) was considered to determine the minimum amount of milk to be used for making of protein, calcium and vitamin B12 source milk drinks.

2. 8. Statistical method

The statistical analyses of the data were performed using one way analysis of variance (ANOVA), with SPSS version 20 and Microsoft office excel. All the data were analyzed in triplicates except for microbial analysis which was analyzed in duplicate Results were expressed as the mean ± standard deviations. To ascertain the significance differences among means of samples, Duncan's multiple range tests was used at p value less than 0.05.

3. Results and discussion

3.1 physicochemical compositions

3.1.1 Ph

In general, the pH levels of milk drinks obtained from markets in Addis Ababa varied between 3.92 ± 0.04 and 4.24 ± 0.03. Notably, there was a statistically significant difference in pH between brand A and brand B, as illustrated in Table 2. Brand A exhibited a pH range of 4.18 ± 0.03 to 4.24 ± 0.03, with an average pH value of 4.22 ± 0.05. In contrast, the pH of brand B ranged from 3.92 ± 0.04 to 3.96 ± 0.07, and the average pH value was 3.95 ± 0.05. Examining various flavors, our investigation revealed that there were not significant differences in pH values attributable to flavor variations for both brands ($p > 0.05$). pH levels below the limits stipulated in the Ethiopian cow milk standard (ESA , 2021), which falls within the range of 6.60 to 6.80.

Our findings indicate lower pH values compared to the results reported by Asefa and Teshome (2019), where the pH of milk collected from Debrezeit and Sebeta milk sheds was noted to be 6.02 and 6.17. This divergence might be attributed to the inclusion of different acids such as citric acid and lactic acid during the preparation of milk drinks, as explicitly mentioned on the labels of the milk drink bottles. Another potential factor could be improper storage conditions for the milk beverages, leading to acid development through lactose fermentation and subsequent reduction in the pH of the milk (Asefa and Teshome, 2019).

3.1.2 Density

As shown in the table below (table 2), the experimental results of density of milk drinks obtained from markets in Addis Ababa varied between 12.14 ± 0.13 and 32.48 ± 0.28 at 20 C^o. Density of brand A milk drinks ranged from 30.65 ± 0.29 to 32.48 ± 0.28, with an average

density value of (32 ± 0.46 g/ mL). In contrast, the density of brand B ranged from 12.14 ± 0.13 to 12.54 ± 0.13, and the average density value was (12.32 ± 0.29 g/ mL). However, the density milk drinks there was not significant differences in density values attributable to flavor variations for both brands ($p > 0.05$) and comparison with Ethiopian cow milk standard brand A milk drinks complain ES but brand B milk drinks not complain Ethiopian cow milk standard.

Our findings indicate that brand B exhibits density values lower to those reported by Bruktawit and Ashenafi, (2016), ranging from 27 g/ mL -35 g/ mL at 16C^{o}. However, brand B shows similar density levels compared to their reported values. This divergence might be attributed to the inclusion of addition of more water during the preparation of milk drinks. Addition of more water was leading to subsequent reduction in the milk density of milk drinks (Bruktawit and Ashenafi, 2016).

3.1.3 Protein

In general, the protein levels of milk drinks obtained from markets in Addis Ababa ranged between 1.27 ± 0.03 and 3.15 ± 0.08. Notably, there was a statistically significant difference in protein between brand A and brand B, as illustrated in Table 2. Brand A exhibited a protein range of 3.03 ± 0.04 to 3.20 ± 0.14, with a significantly higher average protein value of 3.12 ± 0.10. In contrast, the protein of brand B ranged from 1.27 ± 0.03 to 1.3 ± 0.08, and the average protein value was 1.28 ± 0.07. Examining various flavors, our investigation revealed that there were not significant differences in protein values attributable to flavor variations for Brand A ($p > 0.05$).

All milk beverages gathered from the 11 sub-cities exhibited protein levels above the limits stipulated in the Ethiopian milk drink standard (ESA , 2021), which falls within the (1%). Our findings reveal that brand A exhibits protein values similar to those reported by Asefa and Teshome (2019), Keba (2020), and Bruktawit and Ashenafi (2016) for pasteurized milk, ranging from 3.08% to 3.28%, 3.01%, and 3.2%, respectively. However, brand B shows lower protein levels compared to their reported values. In contrast, both brands in our study demonstrate higher protein content than the results documented by Nnubia et al. (2020), who found protein levels in milk drinks commercially available in Nigeria ranging from 0.75 ± 0.16 to 1.31 ± 0.11. This discrepancy suggests that variations in the formulations of milk drinks among brands contribute to a substantial degree of variability in the nutrient composition of these beverages, even when sharing the same flavor base.

3.1.4 Fat

As shown in the table below (table 2), the experimental results of fat of milk drinks obtained from markets in Addis Ababa varied between 0.43 ± 0.09% and 1.19 ± 0.07%. Notably, there was a statistically significant difference in fat between brand A and brand B, as illustrated in Table8. Brand A exhibited a fat range of 0.43 ± 0.09 to 0.57 ± 0.06, with significantly lower average fat value (0.50 ± 0.11%) as compared to brand B. In contrast, the fat of brand B ranged from 1.09 ± 0.11to 1.19 ± 0.07, and the average fat value was 1.13 ± 0.12. Examining various flavors, our investigation revealed that there were not significant differences in fat values attributable to flavor variations for both brands ($p > 0.05$).

All milk beverages gathered from the 11 sub-cities exhibited protein levels brand A fat level below the limits stipulated in the Ethiopian milk drink standard (ESA , 2021) , which falls (1.1%) in contrast brand B complain ES value. Our results indicate decreased values when compared to the findings reported by Keba (2020), who noted a fat content of 3.63% in milk collected from the Oromia region. Furthermore, there is a lower fat content in all milk drinks collected from various sub-cities in comparison to the results documented by Nnubia et al. (2020), where the fat content of commercially sold milk drinks in Nigeria ranged from 1.29 ± 0.07 to 2.37 ± 0.04%. This disparity suggests that the observed difference may be attributed to the addition of more water instead of milk during the preparation of milk drinks by vendors. Another potential factor contributing to the lower fat values could be the removal of cream, as discussed by Adam (2009).

3.1.5 Solid not Fat

All solid not fat (SNF) levels of milk drinks obtained from markets in Addis Ababa varied between 3.47 ± 0.07% and 8.57 ± 0.23%. Notably, there was a statistically significant difference in SNF between brand A and brand B, as illustrated in Table 2. Brand A exhibited a SNF range of 8.24 ± 0.09 to 8.57 ± 0.23, with an average SNF value of 8.43 ± 0.19. In contrast, the SNF of brand B ranged from 3.47 ± 0.07 to 3.58 ± 0.24, and the average SNF value was 3.47± 0.19. It clearly indicating that brand A milk drinks have significantly higher SNF than brand B milk drinks. After examining various flavors, our investigation revealed that there were no statistically significant differences in SNF values linked to flavor variations for both brands ($p > 0.05$).

The values of all the SNF were above the minimum regulatory limit of the Ethiopian milk drink Standards. Our results reveal lower SNF values in brand B and comparable SNF values in brand A when compared to the reported findings by Keba (2020) and Bruktawit and Ashenafi (2016), where the SNF content of milk was documented as 8.20% and 7.6%, respectively. This

discrepancy may be attributed to the removal of milk fat as cream and the addition of inexpensive bulking additives, such as low-quality flour, which contribute to increased total solids (Shehzadi and Khan, 2016). Additionally, other potential factors influencing the nutrient composition of milk drinks include the genetics and breed of the animal, environmental conditions, stage of lactation, parity, and the nutrition of the cow (Jenkins and McGuire, 2006).

Table 2 Physical and Chemical composition of two brands of milk drink samples with four flavors

Brand type	Flavors	Protein	Fat	SNF	pH	Density
A	Mango	3.03 ± 0.04^{b}	0.57 ± 0.06^{a}	8.24 ± 0.09^{b}	4.24 ± 0.03^{b}	30.65 ± 0.29^{b}
	Orange	3.20 ± 0.14^{b}	0.53 ± 0.09^{a}	8.50 ± 0.15^{b}	4.23 ± 0.04^{b}	32.48 ± 0.28^{b}
	Strawberry	3.15 ± 0.08^{b}	0.47 ± 0.13^{a}	8.57 ± 0.23^{b}	4.21 ± 0.09^{b}	31.97 ± 0.87^{b}
	Apple	3.08 ± 0.04^{b}	0.43 ± 0.09^{a}	8.38 ± 0.09^{b}	4.18 ± 0.038^{b}	31.38 ± 0.39^{b}
	Average	3.12±0.075	0.5 ± 0.09	8.42±0.14	4.22±0.05	31.62±0.46
B	Mango	1.3 ± 0.08^{a}	1.09 ± 0.11^{b}	3.36 ± 0.17^{a}	3.95 ± 0.03^{a}	12.54 ± 0.13^{a}
	Orange	1.29 ± 0.07^{a}	1.16 ± 0.14^{b}	3.58 ± 0.24^{a}	3.95±0.06a	12.38 ± 0.36^{a}
	Strawberry	1.27 ± 0.07^{a}	1.10 ± 0.13^{b}	3.48 ± 0.18^{a}	3.96 ± 0.07^{a}	12.22 ± 0.56^{a}
	Apple	1.27 ± 0.03^{a}	1.19 ± 0.07^{b}	3.47 ± 0.07^{a}	3.92 ± 0.04^{a}	12.14 ± 0.13^{a}
	Average	1.28±0.06	1.14±0.11	3.47±0.16	3.95±0.05	12.32±0.29

Results are expressed as Mean ± SD. Means followed by different superscript letters along the column are significantly different ($P<0.05$). SNF= Solid Not-Fat; ES = Ethiopian standard

3.2 Microbial quality

3.2.1 Total plate count

The total plate counts levels of milk drinks obtained from markets in Addis Ababa varied between 0.8 log 10 CFU/ mL to 2.64 log 10 CFU/ mL as indicated in table 3. Brand A's milk drinks displayed an elevated range of total plate counts, spanning from 0.93 log 10 CFU/ mL to 2.64 log 10 CFU/ mL , and an average total plate count value of 1.48 log 10 CFU/ mL . Conversely, brand B's total plate counts were lower, ranging from 0.8 log 10 CFU/ mL to 2.17 log10 CFU/ mL, with an average total plate count value of 1.31 log 10 CFU/ mL.

Our total plate count findings revealed values that were lower than those reported by Ntuli et al (2016), where the total plate counts of milk ranged from 2.2 to 4.8 log 10 CFU/ mL. This variance could be linked to potential cross-contamination during the preparation of milk drinks and milk collection. Another contributing factor might be inadequate storage conditions for the milk beverages, subsequently leading to an increase in the total plate counts of the milk (Koushki et al., 2016). The total microbial count serves as an indicator of the hygienic quality of food products.

3.2.2 Total Coliform count

The total coliform count levels of milk drinks obtained from markets in Addis Ababa varied between 0 log10 CFU/ mL to 1.02 log 10 CFU/ mL as illustrated in Table 3. Brand A milk drinks exhibited higher total plate counts range of 0 log 10 CFU/ mL to 1.02 log CFU/mL, with an average total coliform count value of 0.5 log 10 CFU/ mL. In contrast, the total coliform count of brand B milk drinks had lower ranged from 0 log10 CFU/mL to 0.49 log 10 CFU/ mL, and the average total coliform count value was 0.33 log 10 CFU/ mL.

Our coliform count results indicated lower total coliform count values compared to the results reported by Ntuli *et al*, (2016) and Mikru *et al*, (2021), where the total coliform count of milk was noted to be on the range between 0 to 2.5 log 10 CFU/ mL. This divergence might be attributed to either defect in pasteurization process or post pasteurization contamination which includes contamination in packaging materials, defects in pipe lines (Jamal *et al*., 2018), subsequently increases the total coliform counts of the milk drink As we know that presence of coliform bacteria indicate fecal contamination making the quality of the industry not satisfactory.

3.2.3 Escherichia coli

In the current study, the *E.coli percent* levels of milk drinks obtained from markets in Addis Ababa varied between 0% to 9.09% as illustrated in Table 3. Brand A and brand B milk drinks exhibited similar *E.coli percent* range of 0% to 9.09% with an average *E.coli* value of 4.55%.

Our *E.coli* results indicated lower *E.coli* values compared to the results reported by weldemedhin, (2018) where they reported that *E.coli* on the pasteurized milk was 6.2% and our result was almost near to samples analyzed in South Africa done by Koushki *et al*, (2016) where they reported that *E. coli* percent on the pasteurized milk was 3.9%. The difference could be due to contamination with waste water and fecal materials (Vahedi *et al*, 2013).

S. aureus ,*Salmonella spp* , Yeast and mold*:* - were not detected in all of the studied samples (for both brand A and brand B) regardless of the flavors used for making the milk drinks as shown in table 3 This study was in a clear agreement with the research output done by Aberra, (2010) where they reported that *salmonella* on pasteurized milk was not isolated. The values of the microbial quality, except for the *S. aureus, Salmonella spp* , Yeast and mold, were below the minimum regulatory limit of the Ethiopian milk drink Standards.

Table 3 Microbial quality of milk drinks for the two brands with four flavors

		parameter tested (Log10 CFU/mL)				Absent and present	Percentage
Brand type	Flavors	TPC	TCC	Yeast and mould	*S.aureu*	*Salmonell spp*	*E coli*
A	Mango	1.1	0.49	0	0	-ve	9.09
	Orange	1.3	0.51	0	0	-ve	0
	Strawberry	2.64	1.02	0	0	-ve	9.09
	Apple	0.9	0	0	0	-ve	0
Average		1.48	0.5	0	0		4.55
B	Mango	0.8	0	0	0	-ve	9.09
	Orange	1.3	0.49	0	0	-ve	0
	Strawberry	2.17	0.41	0	0	-ve	0
	Apple	0.9	0.42	0	0	-ve	9.09
Average		1.31	0.33	0	0	-ve	4.55

Total average	1.4	0.42	0	0	Ve	4.55

3.3 Heavy metals

As shown in table 4, the three metals were detected in all milk drink brands and flavors. It is known that the heavy metal contents in milk can vary widely due to many factors such as its secretion from the mammary gland, feeding, use of different water sources and industrial pollutions (Asayehegn *et al*., 2021).

3.3.1 Chromium

In Addis Ababa's markets, the chromium (Cr) levels in milk drinks generally ranged from 0.04 ± 0.02 to 0.18 ± 0.05 mg/L. Notably, a statistically significant difference in Cr was observed between brand A and brand B, as indicated in Table 4. Brand A showed a Cr range of 0.15 ± 0.15 to 0.18 ± 0.05, with an average Cr value of 0.17 ± 0.08. In contrast, brand B's milk drinks had Cr levels ranging from 0.04 ± 0.02 to 0.14 ± 0.02, with an average Cr value of 0.09 ± 0.03. These results clearly demonstrate that, on average, brand A's milk drinks had a significantly higher Cr value (0.17 ± 0.08 mg/L) compared to the average Cr value of brand B's milk drinks (0.09 ± 0.03 mg/L). When considering various flavors, our investigation found no significant differences in Cr values due to flavor variations for both brand A and B ($p > 0.05$).

Our findings align with the research conducted by Alem et al (2015), wherein they reported that the chromium (Cr) concentration in milk closely resembled our results, with a mean value ranging from 0.055 to 0.075 mg/L. Our results indicated higher Cr values compared to the findings reported by Belete et al (2014), Akele et al (2017), and Asayehegn et al (2021), where the Cr levels in milk were noted to be 0.845 to 0.895 mg/L, 0.468 to 0.828 mg/L, and 0.00 mg/L to 0.4 mg/L, respectively. This disparity might be attributed to potential contamination, which could occur incidentally during the use of potable water for cleaning equipment on the farm or in the processing plant (Wang et al, 2016) and during the preparation of milk drinks

3.3.2 Lead

In Addis Ababa's markets, the Lead (Pb) levels in milk drinks generally ranged from 0.38 ± 0.09 to 0.60 ± 0.10 mg/L. Notably, a statistically significant difference in Pb was observed between brand A and brand B, as indicated in Table 4. Brand A showed a Pb range of 0.38 ± 0.09 to 0.48 ± 0.23, with an average Pb value of (0.45± 0.15 mg/L). In contrast, brand B's milk drinks had Pb levels ranging from 0.50 ± 0.16 to 0.60 ± 0.10, with an average Pb value of (0.56 ± 0.12 mg/L).

These results clearly demonstrate that, on average, brand B's milk drinks had a significantly higher Pb value (0.56 ± 0.12mg/L) compared to the average Pb value of brand A's milk drinks (0.45± 0.15mg/L). When considering various flavors, our investigation found no significant differences in Pb values due to flavor variations for both brand A and B ($p > 0.05$).

our findings indicate lower Pb values compared to the finding reported by Abdulkhaliq *et al* (2012) in Palestine, where the Pb of milk was noted to be 0.0 to 0.39 mg/L and higher research output done by Ahmad *et al* (2016) in Bangladesh, where they reported that the Pb concentration of the milk were noted to be 0 – 0.93 mg/L. This divergence might be attributed to the inclusion of tap water during the preparation of milk drinks because; many heavy metals were reported in drinking water (Chowdhury *et al*, 2016). Another potential factor might be consumption of contaminated feeding stuffs and the commonly used drinking water for animals.

3.3.3 Cadmium

In Addis Ababa's markets, the cadmium (Cd) levels in milk drinks generally ranged from 0.94 ± 0.43 to 1.25 ± 0.76 mg/L. Notably, a statistically significant difference in Cd was not observed between brand A and brand B, as indicated in Table 4. Brand A showed a Cd range of 1.08 ± 0.69 to 1.25 ± 0.76, with an average Cd value of 1.16 ± 0.73. In contrast, brand B's milk drinks had Cd levels ranging from 0.94 ± 0.43 to 1.17 ± 0.85, with an average Cd value of 1.02 ± 0.39. These results clearly demonstrate that, on average, brand A's milk drinks had a significantly higher Cd value (1.16 ± 0.73 mg/L) compared to the average Cd value of brand B's milk drinks (1.02 ± 0.39mg/L). When considering various flavors, our investigation found no significant differences in Cd values due to flavor variations for both brand A and B ($p > 0.05$).

our findings indicate lower Cd values compared to the finding reported by Malhat *et al*, (2012) in Egypt, Abou-Arab *et al*, (2008) in Egypt, Abdulkhaliq *et al*, (2012)) in Palestine, where the Cd of milk was noted to be 0.20 to 0.29 mg/L , 0.016 to 0.04 mg/L, 0.022 to 0.057 mg/L respectively. The main source of Cadmium exposure is food. This metal is responsible for a multiplicity of toxic effects on human health (Pastorelli *et al*., 2023). . This is good indicator for monitoring the sanitary conditions practiced during milk collection, production, secondary contamination, and type of packaging. The values of the heavy metals, Pb and Cd of milk drinks was not complain Ethiopian milk drink Standards .similarly Cr was complain the requirement of Ethiopian cow milk.

Table 4 Heavy metals (Cr, Pb and Cd) status of two brands of milk drinks with four flavors

Brand type	Flavors	Cr (mg/L)	Pb (mg/L)	Cd (mg/L)
A	Mango	0.16±0.08[b]	0.46 ± 0.12[a]	1.22±0.93[a]
	Orange	0.18 ± 0.08[b]	0.48 ±0.23[a]	1.08±0.69[a]
	Strawberry	0.15± 0 .15[b]	0.47 ±0.15[a]	1.13±0.84[a]
	Apple	0.18± 0 .05[b]	0.38± 0.09[a]	1.25±0.76[a]
	Average	0.17±0.08[b]	0.45± 0.15[a]	1.16± 0.73[a]
B	Mango	0.05 ± 0.03[a]	0.50 ± 0.16b	1.17±0.85[a]
	Orange	0.13 ±0 .03[a]	0.60 ± 0.10[b]	0.94±0.43[a]
	Strawberry	0.04±0 .02[a]	0.55 ± 0.13[b]	1.11±0.75[a]
	Apple	0.14± 0.02[a]	0.57 ± 0.10[b]	1.11± 0.72[a]
	Average	0.09± 0.03[a]	0.56±0.12[b]	1.02±0.39[a]

Results are expressed as Mean ± SD. Means followed by different superscript letters along the column are significantly different ($P<0.05$).

3.4 Percentage of the main raw materials

As it is clearly depicted in table 5, for the milk drink to be claimed as a source of protein, the calculated amount of whole milk to be used was found to be 81.69% with 18.31% water. Similarly for the milk drink to be claimed as a source of calcium, 49.18% whole milk is required to be mixed with 50.82% water and to be claimed as a source of vitamin B12, 16.67% whole milk was found to be mixed with 83.33% water.

Milk is considered as one of the nutritious food and important for human health, specifically to children. However, its nutritional value could be decreased by the addition of extra water. Consuming milk of poor quality could be harmful to people's health (Asged and El Zubeir, 2021). As per the calculation made using Codex CAc/CL 23 and Codex CAC/GL 2, milk drink should constitute at least 81.69% of whole milk and the remaining around 19% could be water so as to be considered as a source for all of the three nutrients considered in this study (protein, calcium and vitamin B12) as indicated in table 13 below.

Table 5. Calculated percentages of main raw materials (milk and water) of milk drinks to make it protein, calcium and vitamin B12 source.

Nutrients	Nutrient composition of whole cow milk	NRV[b]	Contribution to NRV to be claimed source for the specific nutrient (%)	Calculated amount of whole milk to be added (%)	Calculated amount of water to be added (%)
Protein	3.06(g)	50	5	81.69	18.31
Calcium	122 (mg)	800	7.5	49.18	50.82
Vitamin B12	0.45 (ug)	1	7.5	16.67	83.33

NRV = Nutrient reference value

a: obtained from EPHI food composition table

b: obtained from codex guideline on nutrition labeling (Codex CAC/GL 2)

c: obtained from codex guideline for nutrition and health claims (Codex CAc/CL 23)

4. Conclusions

Results of this study indicated that All physicochemical compositions and heavy metals were significance difference in both brands and also there were not significance difference in flavor variation except chromium. Physicochemical of milk drinks density, PH and Fat content was below the standard in both brands of samples and also Heavy metals Pb and Cd were not complain Ethiopian milk drink standard. Microbial quality of TPC, TCC and *E.coli* was not complaining Ethiopian milk drink standard. The presence of pathogenic organisms and heavy metals is harmful to human health. Milk drink Percentage of raw materials shows higher percentage of cow milk to be added to make minimum protein amount. However, its nutritional value could be decreased by the addition of extra water. Consuming milk of poor quality may be harmful to people's health. Generally it can be conclude that all tested parameters indicates that milk drink available in Addis Ababa are some are good quality and the some are very bad and will cause health risk to consumers.

AUTHOR CONTRIBUTIONS

Banchayhu Getahun: Formal analysis (equal); funding acquisition (equal); investigation (equal); writing – original draft (equal).

Aynadis Tamene: Conceptualization (equal); formal analysis (equal); methodology (equal); resources (equal); supervision (equal); validation (equal); writing – original draft (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

This study was approved by the Institutional Review Board of the College of Natural and Computational Sciences of Addis Ababa University.

5. References

Abdeljalil, N. A., Benkhayal, A. A., Ahmida, M. H. S., El Sharaa, I. A., & Ahmida, N. H. (2021). Determination of Lead and Cadmium in Different Types of Milk Samples Collected from Different Markets in Benghazi-Libya

Abdulkhaliq A, Swaileh K, Hussein M & Matani M (2012). Levels of metals (Cd, Pb, Cu and Fe) in cow's milk, dairy products and hen's eggs from the West Bank, Palestine. *International Food Research Journal*, 19: 1089-1094.

Aberra, A. M. (2010). Microbiological safety of pasteurized and raw milk from milk processing plants in and around Addis Ababa (Doctoral dissertation, Addis Ababa University).

Abou-Arab A, Abou-Donia M & Enb A (2008). Chemical composition, metals content and pesticide residues in raw, pasteurized and UHT milk and their dietary intake. *Journal of the Egyptian Society of Parasitology*, 39: 111-121.

Ahmad M, Roy S, Sarwar N, Morshed S, Alam M, Matin A & Kobra T (2016). Contamination of raw fresh milk, market pasteurized milk and powdered milk by toxic heavy metals in Bangladesh. *Scientific Research Journal*, 4: 19-24.

Akele M, Abebe D, Alemu A, Assefa G & Madhusudhan A (2017). Analysis of trace metal concentrations in raw cow's milk from three dairy farms in North Gondar, Ethiopia: chemometric approach. *Environment Monit Assess*, 3: 499-511.

Alem G, Tesfahun K & Kassa B (2015). Quantitative determination of the level of selected heavy metals in the cows' milk from the Dairy Farm of the Haramaya University, Eastern Ethiopia. *International Journal of Chemical and Natural Science*, 3: 240-248.

Asayehegn, A. T., Hymete, A., Hagos, S., & Ashenef, A. (2021). Determination of the Level of Heavy Metals in Cow's Milk Collected from Butajirra and Meskan Districts, South Central Ethiopia.

Asefa, Z., & Teshome, 2019. Physical properties and chemical compositions of raw cow milk in milk shades around Addis Ababa, Ethiopia. *Journal of Natural Sciences Research*, 9(19), 33-37.

Asged, A. A., & El Zubeir, I. E. M. (2021) Occurrence of adulterants and preservatives in the Milk Sold in Rural areas of Omdurman, Sudan

Ayza, A., & Yilma, Z. (2014). Patterns of milk and milk products adulteration in Boditti town and its surrounding, South Ethiopia. *J Agric Sci*, 4(10), 512-6.

Belete T, Hussen A & Rao M (2014). Determination of concentrations of selected heavy metals in cow's milk: Borena Zone, Ethiopia. *Journal of Health Science*, 4: 105-112

Bolarinwa, I. F., Aruna, T. E., Adejuyitan, J. A., Akintayo, O. A., & Lawal, O. K. (2018). Development and quality evaluation of soy-walnut milk drinks. *International Food Research Journal*, 25(5), 2033-2041

Bruktawit, S., & Ashenafi, M. (2016). Physicochemical properties and microbial quality of cow milk collected from selected subcity of Addis Ababa, Ethiopia. Addis Ababa University, College of veterinary Medicine and Agriculture, Department of Animal Production Studies

Chowdhury, S., Mazumder, M. J., Al-Attas, O., & Husain, T. (2016). Heavy metals in drinking water: occurrences, implications, and future needs in developing countries. *Science of the total Environment*, *569*, 476-488.

ESA 2021 Ethiopian milk drink specification Second edition ICS: 67.100.10 Published by Ethiopian Standards Agency © ESA milk drink Specification

Fusco, V., Chieffi, D., Fanelli, F., Logrieco, A. F., Cho, G. S., Kabisch, J., ... & Franz, C. M. (2020). Microbial quality and safety of milk and milk products in the 21st century. *Comprehensive Reviews in Food Science and Food Safety*, 19(4), 2013-2049.

Getabalew, M., Alemneh, T., & Akeberegn, D. (2019). Dairy production in Ethiopia-existing scenario and constraints. *Biomed J Sci Tech Res*, 16, 12304-9.

Górska-Warsewicz, H., Rejman, K., Laskowski, W., & Czeczotko, M. (2019). Milk and dairy products and their nutritional contribution to the average polish diet. *Nutrients*, 11(8), 1771

Gutierrez, E., Metcalfe, J. J., & Prescott, M. P. (2022). The Relationship between Fluid Milk, Water, and 100% Juice and Health Outcomes among Children and Adolescents. *Nutrients*, 14(9), 1892

Hossin, M. S., Debnath, S., Alam, M. N., Islam, M. S., Miah, M. K., Mollah, M. S. H., & Saeid, A. (2021). Physico-chemical, sensory and microbiological characteristics of strawberry flavored milk under refrigerated storage. *Asian Journal of Dairy and Food Research*, 40(1), 82-87

Islamoğlu, F., Torul, O., Yazici, E., Duran, C., & Selvi, E. K. (2020). Determination of Some Metal Contents of Cow's Milk in Akçabaat District of Trabzon through the ICP-MS Method. *Turkish Journal of Analytical Chemistry*, *2*(2), 62-68.

Jamal, J. B., Akter, S., & Uddin, M. A. (2018). Microbiological quality determination of pasteurized, UHT and flavoured milk sold in Dhaka, Bangladesh. *Stamford Journal of Microbiology*, *8*(1), 1-6.

Jenkins, T. C., & McGuire, M. A. (2006). Major advances in nutrition: impact on milk composition. *Journal of dairy science*, *89*(4), 1302-1310.

Keba Wirtu, A. (2020). Assessment of Dairy Food Environment, Physicochemical Properties, and Microbial Safety of Milk and Cottage Cheese across the Value Chain in Oromia Region, Ethiopia (Doctoral dissertation, Addis Ababa University).

Koushki, M., Koohy-Kamaly, P., Azizkhani, M., & Hadinia, N. (2016). Microbiological quality of pasteurized milk on expiration date in Tehran, Iran. *Journal of dairy science*, *99*(3), 1796-1801

Lemma, H. D., Mengistu, A., Kuma, T., & Kuma, B. (2017). The potential of milk production and consumption for improving nutrition of smallholder dairy households in Ethiopia. *Milk Science International-Milchwissenschaft*, 70(3), 10-16

Lewis, J. 2019. Codex nutrient reference values. Rome. FAO and WHO.

Lodu, c. (2017). determination of selected heavy metals in vegetables and soil along the shore lines of lake hawassa and tikur wuha river (doctoral dissertation).

Malhat F, Hagag M, Saber A & Fayz E (2012). Contamination of cows milk by heavy metal in Egypt. *Bulletin of Environmental Contamination and Toxicology*, 88: 611-613.

Mikru, A., Adane, M., & Dobo, B. (2021). Microbial hazard analysis in the pasteurized milk production value chain at a commercial dairy plant in Hawassa, southern Ethiopia. *Journal of Advances in Dairy Research*, *9*, 254

Nnubia, U. I., Ezeonyeche, C. L., Nnodim, E. J., Okechukwu, F. O., & Joseph, B. C. June 2020 .Effect of sunlight exposure on the nutrient composition of two selected commercial milk drinks. *Journal of Dietitians Association of Nigeria (JDAN)* Volume 11 Number 1, 2635-3326

Ntuli, V., Njage, P. M. K., & Buys, E. M. (2016). Characterization of Escherichia coli and other Enterobacteriaceae in producer-distributor bulk milk. *Journal of dairy science*, *99*(12), 9534-9549.

Pastorelli, G., Michele, S., Marisanna, S., & Everaldo, A. (2023). Milk quality determined using chemical analysis and microwave plasma atomic emission spectrometry as a function of

seasonality in two conventional Italian dairy farms. Acta Scientiarum Polonorum. *Technologia Alimentaria*, *22*(1), 5-12.

Shehzadi, R., Yu-Qing, T., & Khan, M. I. (2016) Composition and Adulteration Analysis of Milk Samples from Ten Different Towns of Lahore

Tabassum, S., & Uddin, M. A. (2016). Microbiological analysis of different flavoured milk samples collected from various areas of Dhaka city, Bangladesh. Bangladesh *Journal of Microbiology*, 33(1-2), 11-15.

Vahedi, M., Nasrolahei, M., Sharif, M., & Mirabi, A. M. (2013). Bacteriological study of raw and unexpired pasteurized cow's milk collected at the dairy farms and super markets in Sari city in 2011. *Journal of preventive medicine and hygiene*, *54*(2), 120.

Weldemedhin, M. S., & Kindie, M. S. (2018). Addis Ababa university college of health school of allied department of medical.

Zebib, H., & Zewdu, A. (2020). Nutritional Quality, Level of Chemical Contaminants and Adulterants in Milk and Dairy Products in Ethiopia: A Review. *Modern Chemistry,* 8(4), 64