ADDIS ABABA UNVERSITY
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Determination of Levels of Major, Minor and Trace Elements in Ethiopian Ouzo Alcoholic Beverage

BY

DEREJE BEKELE

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Determination of Levels of Major, Minor and Trace Elements in Ethiopian Ouzo Alcoholic Beverage

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Dereje Bekele

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Dedication

To my Wife Marshet Tesfu

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Abstract

Determination of Levels of Major, Minor and Trace Elements ions in Ethiopian local Ouzo Alcoholic Beverage

By Dereje Bekele

Advisor: Prof. B. S. Chandravanishi

Alcoholic beverages are any fermented or distilled liquids such as wine, beer, whisky, ouzo, gin, etc. Ouzo is one type of distilled alcoholic beverage with alcoholic content of 40 to 43 % flavored by anethole extracted from anise, star anise or fennel plant. In this study the concentration of selected metals (Na, Ca, Mg, Fe, Zn, Mn, Co, Cr, Pb, Cu, Ni and Cd) in different Ethiopian ouzo brands (Kokeb, National, Liyu Addis, Victoria and Belezaf) sampled from different supermarket in Addis Ababa were analysed. Known volume of ouzo samples were digested by using 2 mL of H_2NO_3 and 5 mL of H_2O_2 for 90 minutes at a temperature of 180 C^0. The levels of the minerals in the digested ouzo samples were analyzed by using flame atomic absorption spectrometer. The following result were recorded in µg/L for each five ouzo brands Na (18194 - 8529), Ca (8330 - 12830) , Mg (1345 – 10977), Fe (942 – 2881), Zn (642 - 2215), Cu (ND<20 - 212), Mn (15 - 225), Cr (54 - 121), Co (ND<50 - 130). Both Ni (<40) and Cd (<5) are below the detection limit of the instrument in all ouzo brands analyzed. The level of Na is the highest in all brands followed by Ca and Mg respectively except for Kokeb ouzo in which the concentration of Mg is higher than Ca. From trace elements analyzed, Zn was found to be highest next to Fe followed by Mn and Co. Generally the levels of metals are higher in Liyu Addis ouzo compared to other brands. All brands are free of heavy metals (Ni and Cd) and contain Pb, Cr and Co in lesser amount.

Key words: Ouzo, FAAS, Major, Minor, Heavy, Elements, Flavor, Alcohol, Beverage, Anethole

1. Introduction

1.1. Common alcoholic beverage consumed in Ethiopia

Alcoholic beverage are any fermented and distilled liquids such as wine, beer, whisky, ouzo, gin, etc. that contain ethyl alcohol or ethanol (CH_3CH_2OH) as intoxicating agent [1]. Fermentation is a widely practiced ancient technology and fermented foods are an essential part of diets in all regions of the world. Traditional fermented beverages are those that are indigenous to a particular area and have been developed by the local people using age-old techniques and locally available raw materials. Early man probably used short time fermented beverages as a safe substitute for water, since the alcohol content is too low in the early stages of fermentation to produce intoxication. Traditional recipes are handed down through generations and are still used for food processing in many developing countries. Early man probably used fermented beverage as a substitute to unsafe water. In the early stage of fermentation the alcohol content is to low to produce an intoxicating effect. The temporary illness resulting from drinking fermented beverage was less severs than those resulting at a time from consumption of contaminated water. It is interesting that primitive man learned the preparation through observation and experience with out a real understanding of the process. Even today the preparation of most traditional alcoholic beverage is still basically family art that is passed from generation to generation orally [2].

However, developing countries cannot continue to depend on historical methods of food processing. Traditional fermentation processes and the potential for their modernization are increasingly attracting the attention of scientists and policy makers as a vital part of food security strategies and commercial use [2]. In Ethiopia, villagers prepare a wide range of traditional fermented foods and beverages from different raw materials such as cereals, ensete, honey, milk, etc. Some of the known Ethiopian traditional fermented foods and beverages are injera, dabo, ambasha, kocho, bulla, ergo, siljo, tella, teji, areki, borde, cheka, shamita, korefe, keribo, bukire, kineto and merissa [3].

Fermentation is a common step in the preparation of Ethiopian traditional alcoholic beverage, but some alcoholic beverage like, araki, redistilled araki, ouzo, etc. requires additional stage that is distillation [4, 5]. Ethiopian ouzo is one of the very popular alcoholic beverages. In many countries there are beverages which either fall outside of the usual beer, wine and spirits categories or which are traditionally produced at the local level, for example in villages and in homes. This kind of production seems especially common in many African countries, where a wide variety of different beverages can be found. Many of these are produced by fermentation of seeds, grains, fruit, and vegetables or from palm trees, which is a rather simple procedure. Through fermentation the alcohol content does not rise very high and often the beverages have a very short shelf life before they are spoilt. Distillation is a more complex procedure requiring more equipment and time, but then the result is both more potent and has a longer shelf life date [6].

Traditional fermented beverage play important roles in social function such as marriage, get together and any other ceremonies. They have also special place in traditional such as burials and settling disputes. In certain parts of African countries they are used as a medicine. Ethiopian traditional beverage consumed on wedding, holydays and other festive occasions in traditional environment. In Ethiopia, well waters, spring waters, lake waters and surface water are used for brewing, sanitation, cooling and boiler feed. In these different sources of water there are different salts and ions in different quantities [7, 8].

1.2. History of ouzo

Ouzo is an alcoholic beverage that contains mainly water, ethanol and trans-anethole. Trans-anethole, an aromatic compound, is showed in Figure 1.

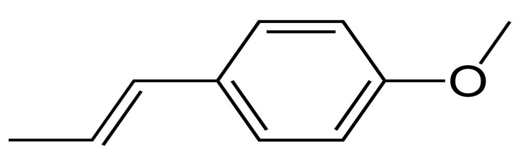


Figure 1. Trans-anethole.

It is extracted from anise, star anise and fennel flowering plant. Anise or aniseed, less commonly anis (stressed on the second syllable) (*Pimpinella anisum*), is a flowering plant in the family Apucea, native to the eastern Mediterranean region and southwest Asia. The leaves at the base of the plant are simple, 2-5 cm long and shallowly lobed, while leaves higher on the stems are feathery pinnate, divided into numerous leaflets. The flowers are white, 3 mm diameter, produced in dense umbels. The fruit is an oblong dry, 3-5 mm long [9].

Anise is sweet and very aromatic. Anise plant is shown in figure 2 below. Aniseed is also used to make the Mexican drink "atole de anis" which is similar to hot chocolate, the Turkish drink raki, the Italian sambuca, the favorite for Arabic arak, some root beer such as virgil's root beer in the United States, and as a digestive after meals in India. It also is used to make the dough, when preparing the famous Peruvian dessert "Picarones".

Figure 2. Anise plant.

Star anise, star aniseed, badiane or Chinese star anise is also a spice that closely resembles anise in flavor, obtained from the star-shaped percarp of *Illicium verum*, a small native evergreen tree of southwest China. The star shaped fruits are harvested just before ripening. It is widely used in Chinese It is widely grown for commercial use in China, India, and most other countries in Asia. Star anise is an ingredient of the traditional five-spice powder of Chinese cooking. It is used as a spice in preparation of biryani in Andhra Pradesh, a south Indian State [10].

Star anise contains anethole, the same ingredient which gives similar to anise in its flavor. Recently, star anise has come into use in the west as a less expensive; substitute for anise in baking as well as in liquor production, most distinctively in the production of the liquor. It is also used in the production of sambuca and pastis.

Figure 3. Star anise fruits (*Illicium verum*).

Fennel (*Foeniculum vulgare* Miller) is also another sample having similar patterns of flavor compositions so used as source of anethole [11].

Fennel (Foeniculum vulgare Miller) is biennial or perennial herb of Umbelliferae up to 2 meters high, with feathery leaves and golden yellow flowers. The fruit (Foeniculi Fructus, Korean; so-hoe-hyang) of fennel is a dry seed from 4-9 mm long, half as wide or less, and grooved. Dried fennel seed is an aromatic, anise-flavored spice, brown or green in color, they slowly turn a dull gray as the seed ages. Fennel seed is used as a flavoring for foods and beverages, and the essential oil from the seed and plant is used in creams, soaps, perfumes, cosmetic, and pharmaceuticals.

Fennel fruits are aromatic, stimulant, and carminative, anti-inflammatory, stimulant and stomachic. Fennel can relieve intestinal gas accumulations and gastro intestinal spasm. Fennel seeds are sometimes confused with the anise seed (Anisi Fructus, *Pimpinella anisum*), which is very similar in taste and appearance, though smaller. In this respect, its use resembled that of anise and dill (*Anethi Fructus, Anethum sowa* or *Anethum graveolens*). In Asia, anise is less known, as fennel and star anise (Illicium verum) being more easily available and more popular. Anise may substitute fennel in Northern Indian recipes, but it is a less suited substitute for star anise in Chinese foods. In China, fennel is found in the well known five-spice powder, along with star anise and cinnamon. China and Vietnam are major producers of trans-anethole from the star anise, and are supplying a large proportion of the demand.

Volatile compounds of fennel**,** star anise and anise seeds extracted by simultaneous distillation-extraction (SDE) and supercritical fluid extraction (SFE) showed similar compositions, with trans-anethole as the main components [12, 13].

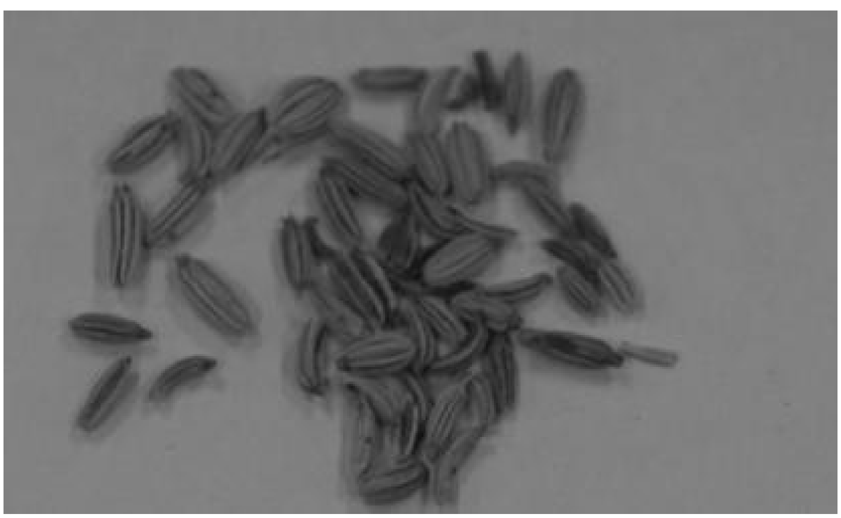


Figure 4. Fennel seed.

1.3. Ouzo consumption and production in the world

Ouzo is a globally famous Greek drink-aperitif. It is made from a precise combination of pressed grapes or raisins and herbs. The distinctive smell of ouzo comes from the addition of anise as flavouring. Modern ouzo distillation largely took off in the 19th century, with much production centred on the island of Lesvos. [10, 13].

The first production line is that of alcoholic drinks. Krinos produces ouzo, brandy, liquor and other alcoholic drinks. Ouzo is a 100% Greek product and Greece's national drink. In the traditionally built cooper distillation flasks the excellent aniseed ouzo is produced according to the traditional secret recipes.

The company exports ouzo and other alcoholic drinks to other European countries. The biggest amount of ouzo sales in Europe is made in Germany where the demand for this traditional Greek drink is quite impressive.

The volume of total consumption of alcoholic drinks shows low rate of growth. In 1999-2003 the annual rate of growth was 6%. The demand is covered mainly by imported products, with the exceptions of ouzo and brandy. The biggest share in the total domestic consumption of alcoholic drinks is that of whisky with percentage 40, 4% in 2003, second in the list is the ouzo with 25, 7%. During the past three-year period (2001-2003), the increase of the consumption of liqueur is remarkable.

1.4. Ouzo making process

Ouzo is a distilled alcoholic beverage. The local traditional method of ouzo making requires the preservation of the ouzo precipitate in the barrel giving a characteristic taste to the ouzo. Once the pure alcohol has been obtained, the producer selects a range of aromatic seeds and grains which he macerates in the alcohol in order to extract all the aromas and flavors that give ouzo its final character. The main flavor component is anise, which provides the core of ouzo's aromatic expression [10, 14].

Even choosing anise is not an easy task, since there are a number of different spices with similar aromas, such as star anise, aniseed, and fennel seed. Their flavor nuances change not only according to type but also according to the spice's age, the degree to which the spice is dried, the quantities used, etc. The final taste is the result of many details: at what stage the spices are added; whether everything is macerated together or separately; and the length of maceration. While anise is the basis of ouzo's flavor, a great deal of ouzo's distinctiveness comes from the skill and creativity the master distiller who uses a range of other herbs and spices. Coriander seeds, fennel seeds, rosemary, even cloves, are among some of the other spices in a typical ouzo recipe. Each producer has his secret recipe, but there are also regional schools of thought. On the island of Chios, for example, where the mastic tree flourishes, ouzo producers spike their mixture with the crystal resin, something that adds a distinct, incense-like aroma to the drink. It is another flavor dimension altogether. The style of ouzo is purely a matter of the producer's preferences. Some ouzos are very sweet; these have been flavored with an additional sweetening agent, such as sugar syrup [10].

Modern method of ouzo making process starts by distilling 96 percent alcohol by volume (ABV) pure ethyl alcohol of agricultural origin (or 96 percent pure ethyl alcohol in which 0.05 percent natural anethole has been added) in copper stills together with anise. Finally sugar may be added and the mix is diluted with water to make ABV from 37.5 to 40%.

1.5. Ouzo consumption and health effect

In the case of ouzo, consumption is almost a ritual. It is the very potent nature of the drink which directly affects how it is served. Ouzo is always served in a small carafe, with ice and water on the side, so that each person can do what he likes with it. One never pours an ouzo for someone else, for example, because each individual prefers to drink it a particular way. Some like it straight up; others on the rocks; others on the rocks and watered down together; others with just a few cubes of ice, which affect the temperature but also slow down the speed with which it is imbibed. People relax as the ice slowly melts and the spirits dilute. The diehard like ouzo in a shot glass, the better to enjoy it in one felt swoop; others want a tall glass, in order to weaken it with lots of water, which helps liberate the aromatic nuances and implies that the drink be sipped and savored slowly [10].

Ouzo is never served alone, but is usually accompanied by food. Matching ouzo with food is an art. It is usually consumed during celebration via on holiday, special occasion, get-together, wedding. etc.

It was reported that the distilled beverages, whiskey, gin and brandy, (which are relatively with high alcoholic content) were conspicuously less poisonous in both sets of experiments than either the wines or malt beverages [16]. Hence it can be concluded that the poisonousness of the alcoholic beverage cannot depend on the alcoholic content and it is occurs due to the presence of other component. In November 1985, the Canadian Government indicated that it had detected ethyl carbamate, $C_3H_7NO_2$, which is developed naturally during the fermentation of alcoholic beverages a suspected carcinogen, in some distilled spirits and wines [17].

The presence of trace metallic ion in alcoholic beverage could be attributed to the so called primary sources (transfer of metal from the soil/groundwater to the grapes and finally to the alcoholic beverage, or atmospheric deposition of airborne particulate matter on grapes) and secondary sources including the making process (clarification and fining gents), in this specific case ouzo processing equipment (valves, pipes, pumps, bronze tanks), and bottles (cork capsules used for sealed bottles), from water used for processing. Some heavy metals are indispensable for us in very small quantities, but become toxic at higher doses [18, 19].

According to Cvetkovic *et al*. waste incineration, phosphate fertilizer manufacture, wood, coal, oil and gasoline combustion, iron and steel production, industrial metal applications and non-ferrous metals mining are also source for metallic ion in alcoholic beverage [20]. Mineral elements can generally be classified as nutritionally essential major elements, such as Ca, K, Mg and Na, nutritionally essential minor and trace elements, like Fe and those regarded as toxic or with an essential/toxic duality: As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Pd, Se, Sn, Tl, V, and Zn all can be exist in alcoholic beverage. At excessive levels, even nutritionally essential elements may exhibit toxicity [21]. If heavy metals present in alcoholic beverage, they may lead to severe effects that include reduced growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death. Exposure to some metals, such as mercury and lead, may also cause development of autoimmunity, in which a person's immune system attacks its own cells. This can lead to joint diseases such as rheumatoid arthritis, and diseases of the kidneys, circulatory system, and nervous system. Manganese, lead and cadmium are recognized as neurotoxic metals [22]. Hence too much intake of alcoholic beverage can expose to health disorder due to the presence these heavy metallic ions.

1.6. Nutritional importance of elements

Food and beverages ingested by humans represent a potentially proficient pathway of exposure to toxic and nutritionally important minor and trace elements. Many mineral elements occur in living tissues, food and diets in such small amounts that they are frequently described as “traces” and the phrase “trace elements” arose to describe them. At the present time, less than one-third of the 90 naturally occurring elements are known to be essential for life. All living things require essential and non-essential elements (C, O, H, K, P, N, Ca, Mg Zn, S, B, Cu, Mn, Mo, Cl and Fe) for their proper growth in certain quantities, These are generally called mineral nutrient [21]. Of these elements C, O and H are derived from air and water; hence they said to be natural mineral nutrient, the rest feed to the organism in different way.

All elements cannot be required in equal percentage. Some are consumed in larger amount, some consumed in limited amount and some are required only in minute or very small amount [22].

Humans require a suite of mineral elements in varying amounts for proper growth, health maintenance and general well being [23, 24]. Plant-derived foods have the potential to serve as dietary sources for all human-essential minerals, and with a well-balanced diet that includes mixed sources of grains, fruits, vegetables, roots and tuber crops.

Trace metal analyses have a gradually increasing importance due to the fact that functions of the trace elements in various areas such as biological systems, environment, and geochemistry are now understood well. Investigations on air, water and soil pollutions, especially metal contamination, for living organisms and/or materials require the determination of the trace metals [25].

Many individuals both in developed and developing countries are failing to attain recommended mineral intakes. Whereas an increased consumption of plant food products would be beneficial, it appears that behavioral and/or environmental factors will continue to limit their consumption [25]. Thus, as an alternative strategy, efforts are underway to increase the nutrient composition of those plant foods which people do eat, as an attempt to ensure adequate attainment of dietary nutrients in all individuals.

Currently, the achievable densities of minerals in our existing agricultural crops means that few individual plant foods are able to supply the daily recommended intake for any given mineral in an average or reasonable serving size. This problem of low mineral density is particularly troublesome in staple foods, such as cereal grains tuber crops and root crops, which make up a large proportion of daily food intake in the developing world [26].

The amount of an element in a plant or animal must not be considered a criterion of its relative value. For some elements present in very small quantities are as essential to growth as those, which compose the greater portion of the plant or animal. The number of elements in a plant is determined by their presence in the soil and air. The amount or proportion depends on many factors: species, edge and root distribution of the plant, physical and chemical nature of the soil, proportion and distribution of the element, method of cultivation, general climatic condition. In an animal, however, the most important single factor is the food, which the animal consumes [22-24, 27].

The concentration of major and minor elements in living tissues can be expressed in grams per kilograms. On the other hand, the concentration of trace elements in living tissues varies between 0.01 and 100 mg kg^{-1}. It may not be appropriate to classify them as essential or toxic elements. It is logically wrong to establish a category of "toxic" elements, because any element may be potentially toxic and this property is a function of concentrations to which humans are exposed. Essentiality of the trace elements is established when a further reduction below the range of tolerable levels, better known as "range of safe and adequate intakes", results in a consistent and reproducible impairment of a physiological function [28].

1.7. Classification of trace elements

All major and minor elements are important; besides that, some of the trace elements, e.g. Cr, Fe, Co, Cu, Zn, Se, Mo and I are essential trace elements; and some of them; Mn, Si, Ni, B, V, and Sn are probably essential trace elements; and further some of them As, Cd, Pb, Al and Hg are considered potentially toxic, some possibly essential elements for animal and human life. Actually all essential elements may also be toxic in animals and humans if ingested at sufficiently high levels and for a long enough period [31, 32].

Essential trace elements are required by man in amounts ranging from 50 μg day^{-1} to 20 mg day^{-1}. The bioavailability of essential elements depends on their chemical form, the composition of the diet and health situation of the individuals. Thus, establishment of the optimum daily requirements and determinations of actual daily intake of essential elements are important problems of trace elements in nutrition [27]. The nutritional value and health effect of some major, trace and toxic elements are described below.

Calcium (Ca)

In all organisms calcium is the most common elements in living things next to oxygen, carbon, hydrogen and nitrogen. Calcium forms a vital part of bone and tooth structure, and is also important as a positive ion (Ca^{2+}) in blood clotting, muscle contraction, and nerve impulse transmission. It also participates in glycogen metabolism [32, 34]. The amount of calcium taken per day will vary with age and for women, with pregnancy, lactation and menopause. The basic recommended daily allowance is 800 milligrams. Long-term calcium deficiency can lead to osteoporosis in which the bone deteriorates and there is an increased risk of fractures. While a lifelong deficit can affect bone and tooth formation, over-retention can cause hypercalcemia (elevated levels of calcium in the blood), impaired kidney function and decreased absorption of other minerals. High calcium intakes or high calcium absorption will contribute to the development of kidney stones. Dairy products, such as milk and cheese, are a well-known source of calcium [33].

Magnesium (Mg)

Magnesium is a co-factor of many coenzyme and is essential to maintain both the acid-alkaline balance in the body and healthy functioning of nerves and muscles (including the heart), as well as to activate enzymes to metabolize blood sugars, proteins and carbohydrates [31]. Magnesium is an essential element in human metabolism and is required for over 300 enzyme reactions, including all reactions requiring adenosine triphosphate. Magnesium is required to regulate cell permeability, and inadequate levels of magnesium will severely affect cardiovascular, neuromuscular, and renal functions. That is muscle twitches, nervousness, abnormal heart beat and disorientation. Excess intake of magnesium causes weakness in people with kidney failure [35].

Iron (Fe)

Iron is an essential trace element for most living organisms. However, its availability is limited by the low solubility of Fe (III) and the ability of intracellular free iron to cause the production of toxic radicals. Iron deficiency is a common and serious nutritional problem that afflicts an estimated 30% of the world's population [42, 43]. Iron carries oxygen to the cells and is necessary for the production of energy, the synthesis of collagen, and the functioning of the immune system. Iron deficiency is common only among children and pre-menopausal women. Great care must be taken not to take too much iron, as excess amounts are stored in the body's tissues and adversely affect the body's immune function, cell growth and heart health [31, 34]. The Australian recommended dietary intake (RDI) is 12–16 mg/d, with 12 mg/d generally considered to be acceptable [37].

The deficiency iron is one of the leading risk factors for disability and death worldwide. It results in anemia which is recognized by its symptom such as low blood iron level, small and red blood cells and low blood hemoglobin values [38]. Iron toxicity usually results from a generic disorder called hemochromatosis.

Manganese (Mn)

Manganese is also one of the essential elements to both plants and animals. It is necessary for normal bone metabolism and important enzyme reactions. It also helps to maintain normal nerve, brain and thyroid function [27]. Dietary manganese deficiency can result in numerous biochemical and structural abnormalities. Deficient animals can be characterized by impaired insulin production, alterations in lipoprotein metabolism, an impaired oxidant defense system, and perturbations in growth factor metabolism. If the deficiency occurs during early development, there can be pronounced skeletal abnormalities and an irreversible ataxia.

Manganese toxicity can also pose a significant health risk. Acute manganese toxicity can result in numerous biochemical pathologies [39].

Copper (Cu)

Small amounts of copper are added to a diet, rats respond by increased growth and hemoglobin generation [41]. As an essential element, copper is required by organisms for a wide range of metabolic processes. Copper could even be considered as a prototype for the emergence of biologically important functional systems. As a malleable metal, copper is widely used by humans for domestic and industrial purposes. Since high levels of copper can be detrimental to organisms, it is very useful in the control of unwanted organisms. Thus, the metal plays many roles in microorganisms, plants, animals and humans. Deficiency of copper causes low white blood cell count and poor growth. Excess intake of copper can cause vomiting, nervous system disorder and Wilson's diseases [42].

Zinc (Zn)

Zinc is an essential element and *in vivo* levels are therefore regulated by most organisms. Estimated ranges of daily dietary intakes of total zinc are 5.6−10 mg/day for infants and children aged 2 months–11 years, 12.3–13.0 mg/day for children aged 12–19 years, and 8.8−14.4 mg/day for adults aged 20–50 years. It is an essential nutrient for humans and animals that is necessary for the function of a large number of metalloenzymes, including alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, leucine aminopeptidase, and superoxide dismutase [44, 45]. Toxic effects of zinc in rodents following short-term oral exposure include weakness, anorexia, anaemia, diminished growth, loss of hair and lowered food utilization, as well as changes in the levels of liver and serum enzymes, morphological and enzymatic changes in the brain, and histological and functional changes in the kidney. The level at which zinc produces no adverse symptoms in rats has been set at about 160 mg/kg body weight. Pancreatic changes were observed in calves exposed to high levels of dietary zinc.

Zinc deficiency in animals is characterized by reduction in growth, cell replication, adverse reproductive effects, adverse developmental effects, which persist after weaning, and reduced immunoresponsiveness [46].

Lead (Pb)

Lead has no physiological function in human body. It is a toxic heavy metal and can affect many organs in the human body. During the last decades, an extensive database has been published providing a direct link between exposure to low levels of lead and mental deficiency in children. Prolonged intake of even low concentrations of lead can cause serious problems for human health. Acute high lead exposure can cause serious physiologic effects, including death or long-term damage to brain function and organ systems. Effects of lead exposure vary according to exposure timing and levels, and other factors, and some effects may be latent [47].

Cadmium (Cd)

Up to this day, it could not be shown that cadmium has any physiological function within the human body [48]. Cadmium is a cumulative nephrotoxicant that is absorbed into the body from dietary sources. The biochemical effects of Cd in humans include interference with enzymatic activity, the ability of interacting with nucleic acids and damaging kidney, hypertension and anosmia (absence of smell) [49, 50]. Cadmium is known to accumulate in the kidney. Chromic effect of this leads to calcium deficiency in the rest of the body, particularly in the skeleton [48, 51].

Sodium (Na)

Sodium is a component of many foodstuffs, for instance of common salt. It is necessary for humans to maintain the balance of the physical fluids system. Sodium is also required for nerve and muscle functioning. Too much sodium can damage our kidneys and increases the chances of high blood pressure. The amount of sodium a person consumes each day varies from individual to individual and from culture to culture; some people get as little as 2 g/day, some as much as 20 grams. Sodium is essential, but controversially surrounds the amount required.

Contact of sodium with water, including perspiration causes the formation of sodium hydroxide fumes, which are highly irritating to skin, eyes, nose and throat. This may cause sneezing and coughing. Very severe exposures may result in difficult breathing, coughing and chemical bronchitis. Contact to the skin may cause itching, tingling, thermal and caustic burns and permanent damage. Contact with eyes may result in permanent damage and loss of sight [52].

Chromium (Cr)

Chromium is a mineral that humans require in trace amounts, although its mechanisms of action in the body and the amounts needed for optimal health are not well defined. It is found primarily in two forms: 1) trivalent (chromium 3+), which is biologically active and found in food, and 2) hexavalent (chromium 6+), a toxic form that results from industrial pollution [53]. The element chromium apparently has a role in maintaining proper carbohydrate and lipid metabolism in mammals. As this role probably involves potentiation of insulin signalling, chromium dietary supplementation has been postulated to potentially have effects on body composition, including reducing fat mass and increasing lean body mass.

Chromium was found to correct glucose intolerance and insulin resistance in deficient animals, two indicators that the body is failing to properly control blood-sugar levels and which are precursors of type 2 diabetes [54].

Nickel (Ni)

Nickel's essentiality in higher organisms is questionable. In the order of abundance in the earth's crust, nickel ranks as the 24th element. Thus, humans are constantly exposed to this ubiquitous element although in variable amounts. Due to its abundance, natural nickel deficiency does not occur, moreover a nickel-deficient diet is difficult to maintain because of nickel's abundance in all types of food. The daily dietary intake of nickel is 25–35 g, and it is more than triple the daily requirement. It was suggested that nickel might have a physiological function in higher organisms. This suggestion resulted from animal experiments with nickel deficient diets. It was reported that nickel depletion in rats resulted in increased prenatal mortality as well as alterations of grooming behavior, liver development, and decreased growth. The growth dependence on nickel was more significant in the second depleted generation. The second depleted generation also showed anemia that manifested in decreased hemoglobin and hematocrit values.

Nickel deficiency impairs the absorption of iron from the intestine. Thus, the concentrations of other metals including iron, copper, and zinc were also decreased in the liver of nickel-depleted animals. Nickel deficiency also results in lowered specific activities of many enzymes involved in carbohydrate and amino-acid metabolism. Nickel-induced alterations in serum and hepatic lipids are similar to those that develop after a moderate iron deficient diet [55].

Cobalt (Co)

Cobalt is considered an essential ultra trace element to the man because it is a fundamental component of vitamin B_{12} (cyanocobalamine), a co-enzyme which takes part on several biological processes. Traces of Co are required for utilization of Fe in the haemoglobin formation and DNA synthesis and its absence can cause anemia. Problems associated to heart, thyroid and lung can be observed if doses higher than 500 mg were ingested [50, 56].

1.8. Purpose and scope

Metallic ion content of different alcoholic beverage was reported from different countries and compared with the standard values. The concentrations of fourteen metal ions were determined in 113 commercial Greek wines by atomic absorption and emission spectroscopy and the values are within acceptable ranges [57]. Taylor *et al*. reported that determination of lead, aluminum and cadmium in wine and lithium and cesium in whisky, vodka and rum by ion coupled plasma atomic emission spectroscopy (ICPAES) [58, 59].

Similarly in Spain levels of copper, zinc, calcium and magnesium were measured in alcoholic beverages (whiskies, gins, rums, liquors, brandies, wines and beers) using flame atomic absorption spectrometry (FAAS) [60]. Mineral concentrations were found to be significantly different. In distilled alcoholic beverages, concentrations measured in rums and brandies were statistically lower than those determined in gins and alcoholic liquors). For Cu, measured concentrations were statistically different for each of the five groups of distilled alcoholic beverages studied. In fermented beverages, Zn, Ca and Mg levels were significantly higher than those concentrations determined in distilled drinks. Contrarily, Cu concentrations were statistically lower. Wines designated as sherry had significantly higher Ca and Mg levels.

Report from Brazil indicates that two toxic metal, chromium and arsenic, are determined in Brazilian sugar cane spirit, Cachaca, (alcoholic content 40%v/v) [61]. Direct determination of Cu, Mn, Pb, and Zn in beer by thermospray flame furnace atomic absorption spectrometry [62]. Galani-Nikolakaki *et al*. investigated elements aluminum, arsenic, cadmium, copper, chromium, iron, lead, manganese, nickel and zinc in Greece wine [63]. The concentrations for all the elements that were determined were almost in all cases, well below the maximum permissible levels by the Greek and the European Union legislation.

The determination of metallic ion in palm wine, popular traditional alcoholic beverage in Africa, from Nigeria was reported [64]. However, no study on the metal contents of

Ethiopian alcoholic beverage has been reported in the literature. Hence it is worthwhile to determine the levels of metals in the Ethiopian ouzo alcoholic beverage.

In this work the levels of major and minor elements such as Ca, Mg, K, Na, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in Ethiopian local gin "ouzo" is determined. There are different brands of this common local alcohol beverage, for this particular study only five different brands were selected, namely Liyu Addis, National, Belezaf, Kokeb and Victoria. Except Belezafi which is produced around Addis Ababa, at Sebeta all are produced in Addis Ababa. Liyu Addis at Kotebe, Asneke around Seris, Belezaf at Mekanisa and Victoria at the Akaki. These five different brands of Ethiopian local ouzo beverage for the analysis are selected randomly.

The determination of these metals in ouzo is relevant because they might be essential or toxic to the human body. There is no all agreed standard concentration of metallic ion in ouzo local alcoholic beverage in Ethiopia so it is necessary to determine the levels of some major and trace metallic ion due to toxicity of certain metal in case of excess intake and the value should be compared with other international alcoholic beverage. In this specific study metal ion such as Na^{+}, Ca^{2+}, Mg^{2+}, K^{+}, Cu^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+}, Cr^{3+}, Pb^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+} in ouzo alcoholic beverage were investigated.

1.9. OBJECTIVE

1.9.1. General Objective

- The general objective of this study is to determine metal contents of Ethiopian ouzo alcoholic drink and compares the results with the standard values and levels in other international alcoholic beverage.

1.9.2. Specific Objectives

The specific objectives of this project include:

- To determine the levels of selected metal ions (Na^{+}, Ca^{2+}, Mg^{2+}, K^{+}, Cu^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+}, Cr^{3+}, Pb^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+}) in ouzo beverage (Belezaf, Kokeb, National, Liyu Addis and Victoria).

- To compare the levels of metal ions in the ouzo alcoholic beverage with other alcoholic beverage of Africa and European countries.
- To compare the levels of metal ions in Ethiopian ouzo alcoholic drink.

2. Experimental

2.1. Equipments and reagents

2.1.1. Equipments

A 100 mL pipette was used to measure the ouzo sample during bulk preparation. 10 mL of measuring cylinder was used to measure ouzo and 2 mL and 5 mL pipette for measuring the reagents to prepare sample for digestion. A 250 mL round bottomed flasks fitted with reflux condensers were used in Kjeldahl apparatus hot plate to digest the mixed ouzo samples. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested sample till analysis. BCK SCIENTIFIC MODEL 210 VGP (East Norwalk, USA) flame atomic absorption spectrophotometer equipped with deuterium ark back ground correctors was used for analysis of the analyte metals (Na, Mg, Mn, Ni, Pb, Ca, Fe, Zn, Cu, Co, Cr, Cd) using air-acetylene flame.

2.1.2. Reagents and chemicals

Reagents that were used in the analysis were all analytical grade. HNO_3 (69-72 %) (Spectrosol, BDH, England) and 30% H_2O_2 (BDH Chemicals limited, Poole, England) were used for digestion of ouzo sample. Lanthanum nitrate hydrate (98%, Aldrich, Muwaukee, USA) was used to avoid refractory interference (for realizing calcium and magnesium from their phosphates). Stock standard solutions containing 1000 mg/L in 2% of HNO_3, the metals Na, Mg, Mn, Ni, Pb, Ca, Fe, Zn, Cu, Co, Cr and Cd, (BUCK SCIENTIFIC PURO-GRAPHIC^{tm}) were used for preparation of calibration standards and in the spiking experiments. Deionized water was used throughout the experiment for sample preparation, dilution and rinsing apparatus prior to analysis.

2.2. Procedures

2.2.1. Cleaning apparatus

Apparatus such as volumetric flasks, measuring cylinders and digestion flasks were washed with detergents and tap water, rinsed with deionized water, soaked in 50 % nitric acid for two days, rinsed with deionized water, dried at room temperature and kept in dust free place until analysis begins.

2.2.2. Sampling and sample preparation

Five brand of different ouzo beverage; namely, National, Belezaf, Kokeb, Victoria and Liyu Addis were collected from different supermarket and factories in Addis Ababa. Ouzo alcoholic beverage is sold in a sealed glass bottle. For the analysis five bottles (each containing 700 mL) of each brand was purchased from different direction of Addis Ababa. For sample collection the supermarkets were selected randomly. The collected sample was kept in a laboratory safely and neat. 100 mL of ouzo beverage was taken from each of the five bottles to prepare 500 mL of bulk sample. The prepared bulk sample was kept in a refrigerator until digestion time. Finally 10 mL of ouzo alcoholic beverage was taken from each bulk for the digestion.

2.2.3. Optimization of working procedure

Even though the ouzo sample is color less solution sample matrix should be break down to avoid interference during analysis by flame atomic absorption spectroscopy. To do these different ouzo digestion procedures were tried using the HNO_3, H_2SO_4 H_3PO_4 and H_2O_2 under different volume and reagent, digestion temperature and different digestion time.

2.2.4. Digestion of ouzo sample

Digestion for the ouzo sample was done using the optimized procedure. Exactly 10 mL of ouzo sample was measured using 10 mL measuring cylinder and poured to a 250 mL round bottom digestion flask. 2 mL of H_2NO_3 and 5 mL of H_2O_2 were measured using 2 mL and 5 mL pipette respectively and poured to digestion flask containing 10 mL of ouzo sample and the mixture was digested on a micro Kjeldahl digestion apparatus by setting the temperature to dial at 6 (180 ^{0}C) for 90 min then after the digested solution was allowed to cool for 10 min without dismantling the condenser from the flask and for 10 min after removing the condenser. The cooled solution has been filtered with Whatman®, (110 mm, diam), filter paper then the round bottom flask was rinsed subsequently with 5 mL deionized water. The filtrate was collected in a 50 mL volumetric flask and the solution was diluted to the mark (50 mL) with deionized water. Triplicate digestions were carried out for each bulk sample. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solutions were determined by flame atomic absorption spectroscopy. Six blank solutions, which were prepared by diluting 96% of ethanol to 41% by deionized water, were prepared following the same digestion procedure as the sample.

2.2.5. Determinations of major, minor and trace elements in ouzo samples

10 mg/L of intermediate standard solutions of metals of interest were prepared from1000 mg/L of the atomic absorption spectroscopy standard stock solutions. Each of this intermediate solution was then diluted with deionized water to obtain four working standards for each metal ion. Twelve macro and micro elements; Na, Ca, Mg, Mn, Cd, Co, Cr, Zn, Ni, Pb, Fe, and Cu were analyzed with FAAS (BUCK SCIENTIFIC MODEL 210GP) equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument. Three replicate determinations were carried out on each sample. The same analytical procedure was followed to determine the

metallic ions in six digested blank solutions. Table 1 gives the operating conditions in FAAS followed in analysis of ouzo sample.

Element	Wavelength (nm)	Detection limit (μg/L)	Slit width (nm)	Lamp current (mA)	Energy
Na	589.0	2	0.2	2.0	3.249
Ca	422.9	10	0.7	2.0	3.699
Mg	285.2	1	0.7	1.0	3.983
Cu	324.7	20	0.7	1.5	3.799
Zn	213.9	5	0.7	2.0	3.067
Mn	279.5	1	0.7	3.0	3.930
Ni	232.0	40	0.2	7.0	3.660
Fe	248.3	30	0.2	7.0	3.003
Co	240.7	50	0.2	2.5	2.752
Cr	357.9	50	0.7	2.0	3.680
Cd	228.9	5	0.7	2.0	3.157
Pb	283.2	100	0.7	2.0	3.478

Table 1. Instrumental operating condition in analysis of metals in ouzo samples using flame atomic absorption spectrophotometer.

2.2.6. Recovery test

The efficiency of the optimized procedure was checked by adding known concentration of each metal in 10 mL sample that is spiking. The following is procedure followed for spiking:

3000 µg of 1000 mg/L Na, 300 µg of Zn, 5 µg of Mn and 10 µg of Co and Cr of 1000 mg/L were spiked at once in to 10 mL of ouzo sample and the remaining metals (2000 µg of Na and Ca, 300 µg Fe and 100 µg Pb and 45 µg Cu of 1000 mg/L were spiked at once in to another round bottomed flask containing 10 mLof ouzo sample. 5 mL of H_2O_2 and 2 mL of HNO_3 were added to both spiked samples and the same digestion process as sample was followed. Each sample was determined for their respective spiked metals by atomic absorption spectrophotometer. Each recovery test was performed in triplicates.

2.2.7. Method detection limit

Six blank samples were digested following the same procedure as the samples and each of the samples were determined for the elements of interest (Na, Ca, Mg, Mn, Cd, Co, Cr, Zn, Ni, Pb, Fe, and Cu) by atomic absorption spectrophotometer. The standard deviation for each element was calculated from the six blank solutions to determine method detection limit.

3. Results and Discussion

3.1 Optimization of digestion procedure

The various techniques for elemental determination do not all requires the same degree of sample matrix break down. The choice of a digestion technique should take into account the objective of the final determination and factors such as the matrix composition, the element contents, the possible interferences, the risk of loses and contaminations, the practicality and possible safety hazards in the laboratory [30, 31].

The purposes of sample decomposition are:

- ❖ Converting all the species in which a given element is present in such a way that it becomes present in one defined form.
- ❖ Eliminating interfering substances from the matrix.
- ❖ Obtaining the element in a homogeneous and easily accessible matrix

Different ouzo digestion procedure was tried using the HNO_3, H_2SO_4, H_3PO_4 and H_2O_2 under different volume, reagent, digestion temperature and different digestion time to get optimum procedure for digestion. Table 2 shows different procedure for digestion of ouzo sample to get optimum procedure.

Table 2. Different trials during optimization procedure for ouzo alcoholic beverage.

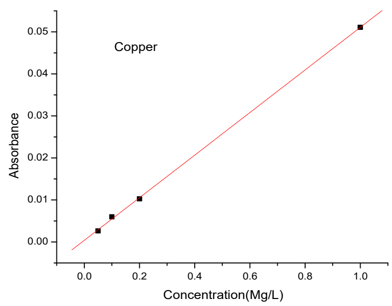
No	Volume of ouzo alcoholic beverage (mL)	Volume of 5:2 HNO_3, $HClO_4$ or H_2SO_4 & H_2O_2 (mL)	Digestion temperature (^0C)	Digestion time (min)	Observation
1	25	7(5:2 H_2O_2 & HNO_3)	120	180	Yellow clear solution with no suspended matter
2	25	7(5:2 $HClO_4$ & HNO_3)	120	180	Brown solution with suspended matter
3	25	7(5:2 H_2SO_4 & HNO_3)	120	180	Clear yellow solution with no suspended matter
4	50	7(5:2 H_2O_2 & HNO_3)	180	180	Clear yellowish solution with no suspended matt
5	50	7(5:2 $HClO_4$ & HNO_3)	180	180	Clear yellowish solution with no suspended matter
6	50	7(5:2 H_2SO_4 & HNO_3	180	180	Clear yellowish solution with no suspended matter
7	**10**	**7(5:2 H_2O_2 & HNO_3)**	**180**	**90**	**Clear colorless solution with no suspended matter**
8	10	7(5:2 $HClO_4$ & HNO_3)	180	90	Deep yellow solution with no suspended matter
9	10	7(5:2 H_2SO_4& HNO_3)	180	90	Deep brown solution with suspended matter
10	25	7(5:2 H_2O_2 & HNO_3)	180	180	Clear light yellowish solution with no suspended matt
11	25	7(5:2 $HClO_4$ & HNO_3)	180	180	Light brown solution with suspended matter
12	25	7(5:2 H_2SO_4 & HNO_3)	180	180	Deep brown solution with suspended matter

Procedure 7, which involves addition of 2 mL of HNO_3 and 5 mL H_2O_2 to 10 mL of ouzo sample and heating at 180 ^0C for 90 min result in clear solution with no suspended matter hence found to be the optimum procedure. This means this step involves (i) minimum reagent volume consumption, (ii) minimum digestion time, (iii) minimum residue and (iv) simple.

All the digestion of ouzo sample and blanks were done following this chosen single procedure.

3.2. Instrument calibration

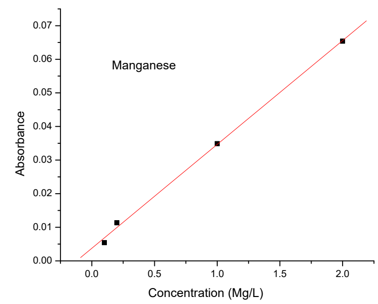
For calibration curves a series of four working standard solution was prepared from 10 mg/L of intermediate solution for each metal ion of interest to be analyzed. The calibration graphs of each of metals of interest are shown in Figure 5 and concentrations of the intermediate standards, working standards and value of correlation coefficient of the calibration graph for each of the metals are listed in Table 3.



Y = 0.000378325+ 0.05069X

R = 0.99986

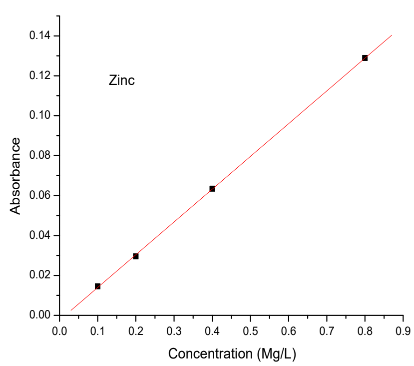
Figure 5a. Calibration graph of copper standard solution .



Y = 0.00377+ 0.0309X

R = 0.99909

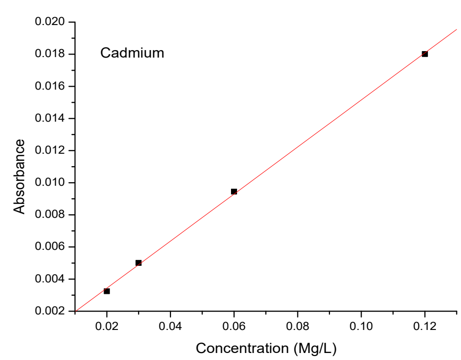
Figure 5b. Calibration graph of manganese standard solution.



Y = -0.00246 + 0.16413X

R = 0.99993

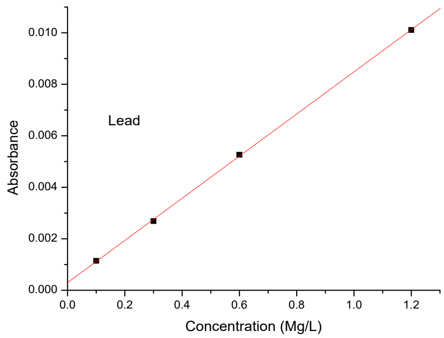
Figure 5c. Calibration graph of zinc standard solution.



Y = 0.000503605 + 0.14649X

R = 0.99971

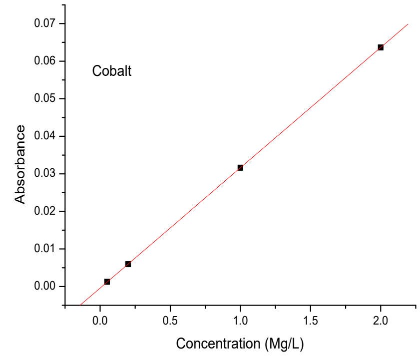
Figure 5d Calibration graph of cadmium standard solution.



Y = -0.000404069+ 0.03202X

R = 0.99999

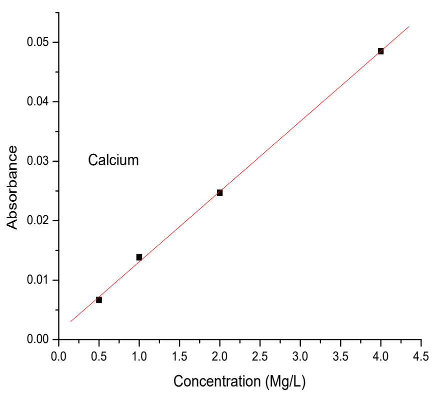
Figure 5e. Calibration graph of cobalt standard solution.



Y = 0.00029971 + 0.00819X

R = 0.99991

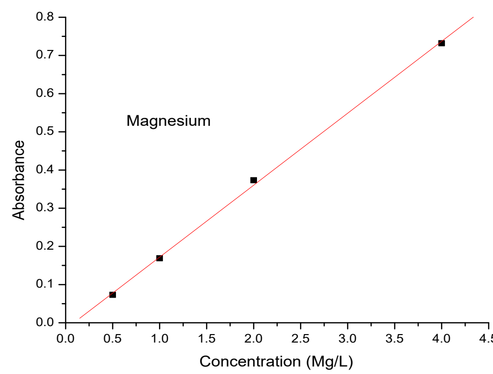
Figure 5f. Calibration graph of lead standard solution.



Y = 0.00128 + 0.01181X

R = 0.99956

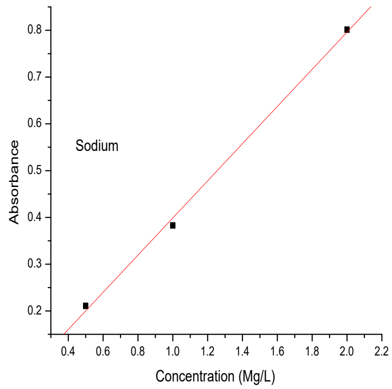
Figure 5g. Calibration graph of calcium standard solution.

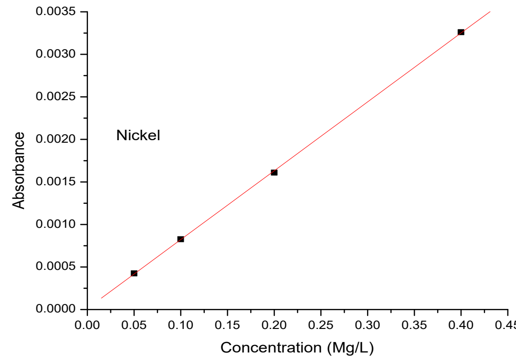


Y = -0.01649 + 0.1884X

R = 0.99956

Figure 5h. Calibration graph of magnesium standard solution.





Y = 0.00138 + 0.39738X

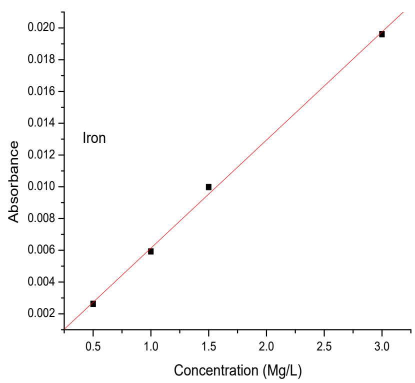
R = 0.99893

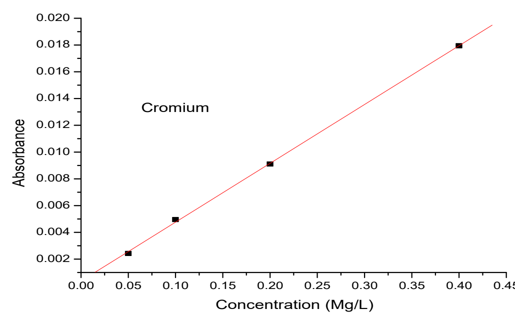
Figure 5i. Calibration graph of sodium standard solution.

Y = 0.0000120435+ 0.0081X

R = 0.99993

Figure 5j. Calibration graph of nickel standard solution.





Y = -0.000675071 + 0.00681X

R = 0.99917

Figure 5k.Calibration graph of iron standard solution.

Y = 0.000364391 + 0.04397X

R = 0.99978

Figure 5l. Calibration graph of chromium standard solution.

Figure 5(a-l). Calibration graph of metals' standard solution.

Table 3. Working standards and correlation coefficients of the calibration curves for determinations of metals using flame atomic absorption spectrophotometer.

No	Metal	Wavelength (nm)	Concentration of intermediate standard (μg/L)	Concentration of standards, in μg/L	Correlation coefficient of calibration curve
1	Na	589.0	10000	500, 1000, 2000, 4000	0.99893
2	Ca	422.7	10000	500, 1000, 2000, 4000	0.99956
3	Mg	285.2	10000	500, 1000, 2000, 4000	0.99956
4	Fe	248.3	10000	500, 1000, 1500, 3000	0.99917
5	Cu	324.7	10000	50, 100, 200, 1000	0.99986
6	Co	240.7	10000	50, 200, 1000, 2000	0.99999
7	Cd	228.9	10000	20, 30, 60, 120	0.99971
8	Pb	283.2	10000	100, 300, 600, 1200	0.99991
9	Cr	357.9	10000	50, 100, 200, 400	0.99978
10	Mn	279.5	10000	100, 200, 1000, 2000	0.99909
11	Ni	232.0	10000	50, 100, 200, 400	0.99993
12	Zn	213.9	10000	100, 200, 400, 800	0.99993

3.3. Evaluation of analytical figures

3.3.1. Precision

Precision is the closeness of agreement between independent test results obtained under stipulated conditions. The precision of the method should be characterised by the repeatability, and the internal reproducibility, which is a measure of intermediate precision obtained over a longer time period within the same laboratory. Repeatability is assessed in terms of within-batch or within-run precision data, while for internal reproducibility, between-batch or between-run precision data.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements [65, 66].

In this study the precision of the results were evaluated by the pooled standard deviation and relative standard deviation of the results of three samples (n = 3) and triplicate readings for each sample meaning that a total of 9 measurements for a given bulk sample. These parameters are useful in estimating and reporting the probable size of indeterminate error. The results of the present analysis are reported with corresponding pooled standard deviation of nine measurements for a bulk sample and triplicate reading per sample and relative standard deviation. The standard deviation and % RSD of each metal in each ouzo sample is shown in Table11.

3.3.2. Limit of detection

The limit of detection is the smallest amount or concentration of analyte in the test sample that can be reliably distinguished, with stated significance, from the background or blank level. It is less important to state the detection limit when evaluating methods for the determination of principal components of foods, than when carrying out analyses at trace level. Even in the latter situation, however, it may not always be considered necessary to quote a detection limit in cases where measurements are being made above the limit of quantitation, which is itself stated as a validated performance characteristic. Detection limit can be made based on 3 times the standard deviation of the concentration in a matrix blank [67].

For present study, six blank samples were digested following the same procedure as the samples and each of the samples were determined for the elements and the pooled standard deviation of the six blank reagents was calculated. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank by three.

3.3.3. Evaluation of analytical methods

Method validation is the process of providing that analytical method is acceptable for its intended purpose. Therefore analysts are increasingly impelled to validate analytical procedures and to estimate uncertainty associated to the results. In this study since there was no certified reference material (CRM) of ouzo in our laboratory, the validity of the optimized digestion procedure for ouzo is checked by carrying out with traceability, such as spiked samples.

Recovery (symbol *R*) is at present used to indicate the yield of an analyte in a preconcentration stage in an analytical method:

$$R_A = \frac{Q_A(\text{yield})}{Q_A(\text{orig})} \times 100 \qquad (1)$$

Where Q_A (orig) is the known original and Q_A(yield) the recovered quantity of the analyte A. If RA is measured using a standard addition or spike procedure,

$$R_A = \frac{Q_A(O + S) - Q_A(O)}{Q_A(S)} \times 100 \qquad (2)$$

Where $Q_A(S)$ is the quantity of analyte A added (spike value) and $Q_A(O + S)$ the quantity of A recovered from the spiked sample and $Q_A(O)$ from the original sample. In all uses of spiked or standard addition procedures sample. The concentration of analyte in the spike should be sufficiently high so as to minimize disturbance, by dilution, of the matrix [68].

Table 4 recovery test results of ouzo samples

Metal	[a]Conc. in sample µg/L	Amount added µg/L	[b]Conc. in spiked sample µg/L	% Recovery
Na	17711 ± 36	3000	20635 ±104	97.5 ± 6.3
Mg	10208 ± 45	2000	12166.3 ± 28	97.9 ±4
Ca	8834 ± 73	2000	10882 ± 90	102.4 ±0.9
Fe	1010 ± 68	300	1338 ± 38	109.2 ±6.3
Zn	679 ± 20	300	996 ± 17	105.7 ±9
Pb	507 ± 4.2	100	627 ± 5.8	109 ±2
Mn	15.3 ± 0.2	5	21 ± 0.3	94.4 ±5.2
Cr	54.4 ± 0.4	10	71 ± 1	110.7 ±9.3
Cu	212 ± 20	45	255 ± 4	96.9 ±4.6
Ni	ND(<40)	—	—	—
Cd	ND(<5)		—	—
Co	ND(<50)	—	—	—

[a]values are average value of triplicate sample and four to five reading in µg/L.
[b]values are mean ± SD of triplicate readings of triplicate analyses in µg/L.

As can be seen from Table 5 the percentage of the recovery test is between 96.9 and 111. It indicates that it is within acceptable range.

3.4 Concentration of major, minor and trace metals in different ouzo brands

The total metal content of five different ouzo brands is not exactly the same. Actually the metal concentration varies based on the source of metal. All manufacturers will not use the same raw material with the same concentration. Leave alone other, water treatment procedure is differing from one to other. Thus the metal concentration may somewhat varied. Even though the metal concentration of each brands differ the order of increasing or decreasing of levels of metal are almost the same. All ouzo brands contain major elements in higher concentration and minor elements in lower quantity. Also some non-essential and toxic metal are below detection limit of the instrument. This indicate that Ethiopian ouzo beverage contain major and essential elements in appreciable amount, minor elements in lower amount and do not contain non-essential and toxic elements. The concentration of 12 metals in each of five different ouzo brands are reported and discussed one by one in the following sub sections.

3.4.1. Concentration of metals in Kokeb ouzo

The concentrations of the metals in the Kokeb ouzo alcoholic beverage are given in Table 5 below. The results indicate that all major elements are found in larger amount and essential trace elements in lower amount. Out of twelve analyzed metals Na is found to be with the largest concentration 17711 ± 36 µg/L followed by Mg which is 10208 ± 45 µg/L and Ca is 8834 ± 73 which is the third appreciable concentration. Compared to Na, Ca and Mg the concentration of heavy metals in Kokeb ouzo is lower. Of these trace metal Co is with higher concentration which is 27.5 ± 2 µg/L followed by Mn 15.3 ± 0.15 µg/L. Kokeb contains higher amount of non-essential metal compared to essential trace elements. Pb is in higher amount 507 ±4.2 µg/L then Cu 212 ± 20 µg/L and Cr is the least 54.4 ± 0.35 µg/L. Other non essential heavy metals, Co (<50), Ni (<40) and Cd (<5) are found to be below detection limit. Figure 6 and 7 display the levels of major and heavy metals in Kokeb ouzo.

Table 5 Levels of metals in Kokeb ouzo beverage

Metal	Na	Mg	Ca	Fe	Zn	Co
Concentration(μg/L)	17711± 36	10208 ± 45	8834 ± 73	1010 ± 68	679 ± 20	-

Metal	Pb	Mn	Cr	Cu	Ni	Cd
Concentration(μg/L)	507 ± 4.2	15.3 ± 0.2	54.4 ± 0.35	212 ± 20	-	-

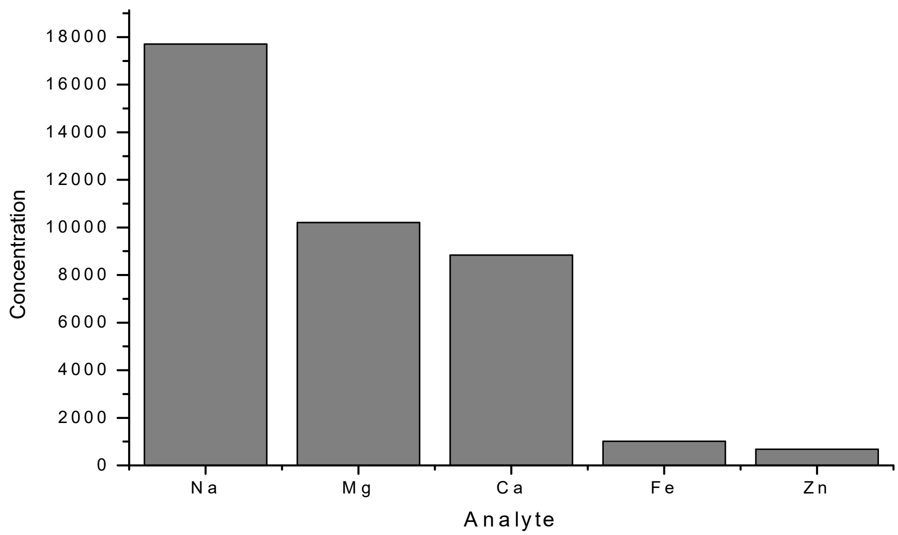


Figure 6. Concentrations of Na, Ca, Mg, Fe, and Zn in Kokeb ouzo in μg/L.

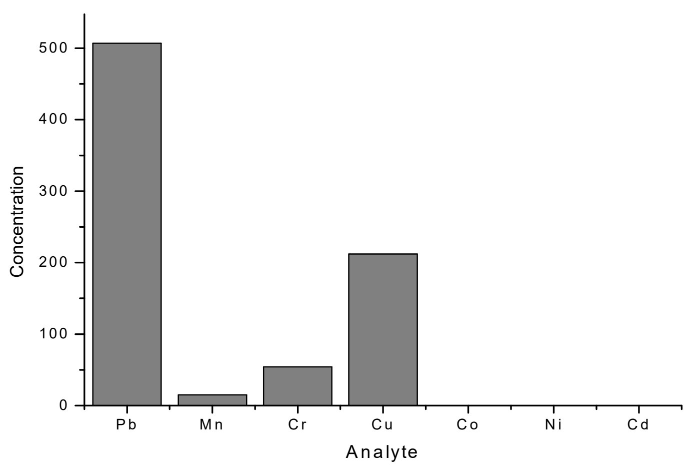


Figure 7. Concentrations of Co, Pb, Mn, Cr and Cu in Kokeb ouzo in μg/L.

3.4.2. Concentration of metals in National ouzo

Compared to other brands National ouzo contains lesser amount of major, minor and trace elements. Table 6 presents concentration of twelve metals in National ouzo. The levels of metals are shown as bar graphs in Figure 8 and 9. The results indicate that the major and some essential trace elements are found in higher amount and non-essential are in lower amount or none. Na is with the highest level 1079 ± 34 μg/L followed by Ca and Mg which is 8330 ± 25 μg/L and 1345 ± 17 μg/L thus ranked as 2nd and 3rd respectively. Of essential trace elements Fe 9429 ± 10 μg/L is the leading concentration followed by Zn 642 ± 20 μg/L then Mn 130 ± 0.53 μg/L. It also contains lesser Pb, Cr Co which is 127 ± 5.6 μg/L, 120 ± 0.3 μg/L and Co (<50), Cu (<20), Ni (<40) and Cd (<5) are below detection limit of the instrument. The concentration of detected elements can be ranked as Na > Ca > Mg > Fe > Zn > Mn > Pb > Cr in increasing order.

Table 6 Levels of metals in National ouzo beverage

Metal	Na	Mg	Ca	Fe	Zn	Co
Concentration (μg/L)	1079 ± 34	1345 ±17	8330 ± 25	942 ± 10	642 ± 20	-

Metal	Pb	Mn	Cr	Cu	Ni	Cd
Concentration (μg/L)	127 ± 5.6	130 ± 0.5	120 ± 0.3	-	-	-

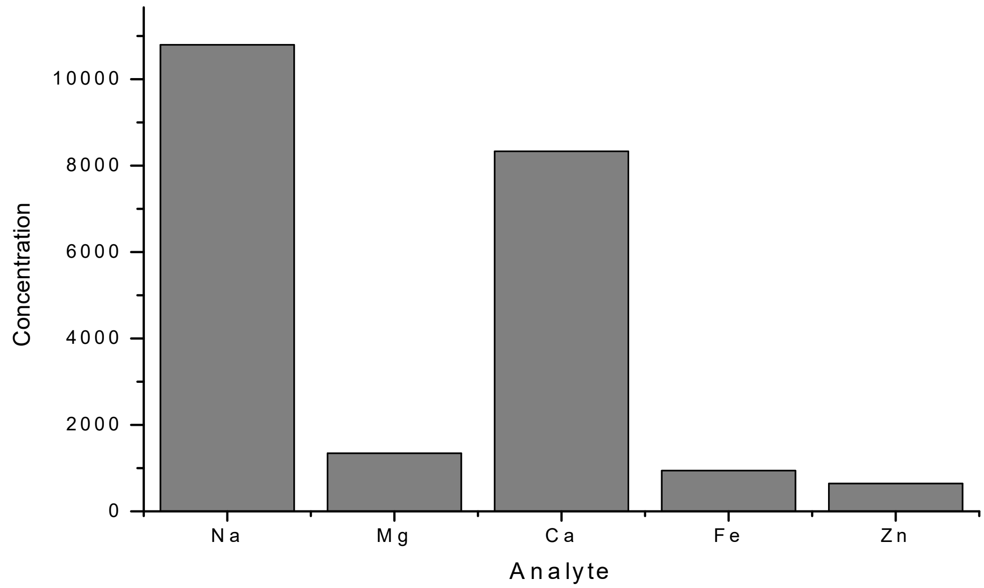


Figure 8. Concentrations of Na, Ca, Mg, Fe, and Zn in National ouzo in µg/L.

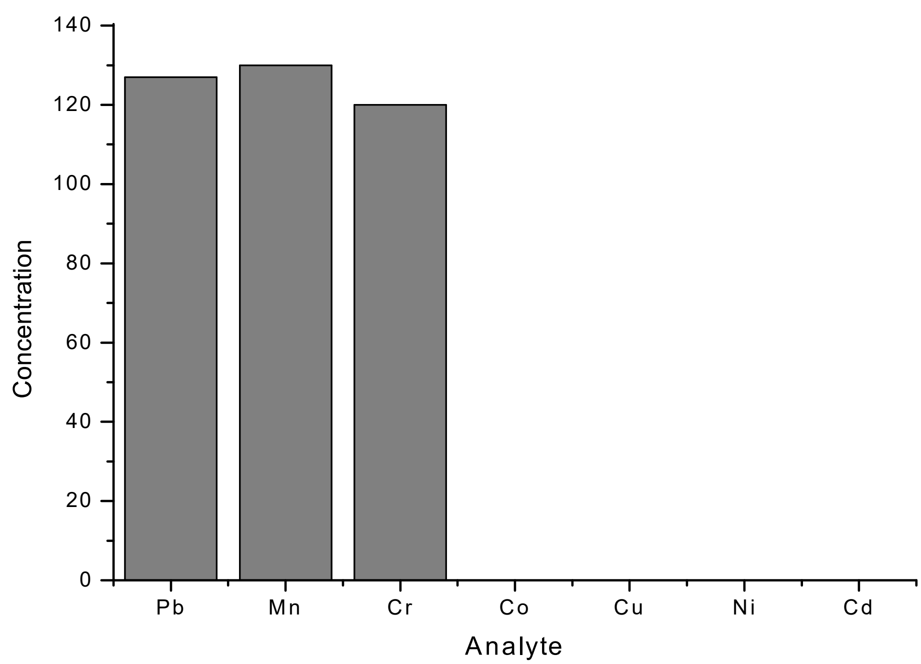


Figure 9. Concentrations of Co, Pb, Mn, Cr, Cu, Ni and Cd in National ouzo in µg/L.

3.4.3. Concentration of metals in Victoria ouzo

Victoria is another brand of ouzo included in this study. Table 7 shows the concentration of major and minor elements. The concentration of metal in this brand is with same trend with the previous two brands. Essential elements exist in higher concentration compared to trace metals. Sodium is with the highest concentration 8529 ± 17 μg/L, Next is Ca and Mg which is 10583± 9 μg/L and 2140 ± 10 μg/L respectively. Essential trace metal still found in lesser concentration compared to major elements. Fe is with higher concentration 1346 ± 39 μg/L followed by Zn 660 ± 3.4 μg/L and Mn 86 ± 0.2 μg/L. Of these non-essential trace metals still Pb is with leading concentration 167 ± 1.5 μg/L followed by Co and Cr which is 123 ± 2.3 μg/L and 121 ± 1 μg/L respectively. Cu (<2), Ni (<40) and Cd (<5) are found to be below detection limit. The concentration trend of analyzed metals in this brand also can be arranged as Na > Ca > Mg > Fe > Zn > Pb > Co > Cr > Mn in their increasing order. Figure 10 and 11 describe the concentration of these metals in bar graph.

Table 7 Levels of metals in Victoria ouzo beverage

Metal	Na	Mg	Ca	Fe	Zn	Co
Concentration (μg/L)	8529 ± 17	2140 ± 10	10583 ± 9	1346 ± 39	660 ±3.4	123.4 ±2.3

Metal	Pb	Mn	Cr	Cu	Ni	Cd
Concentration (μg/L)	167 ±1.5	86 ± 0.2	121 ± 1	-	-	-

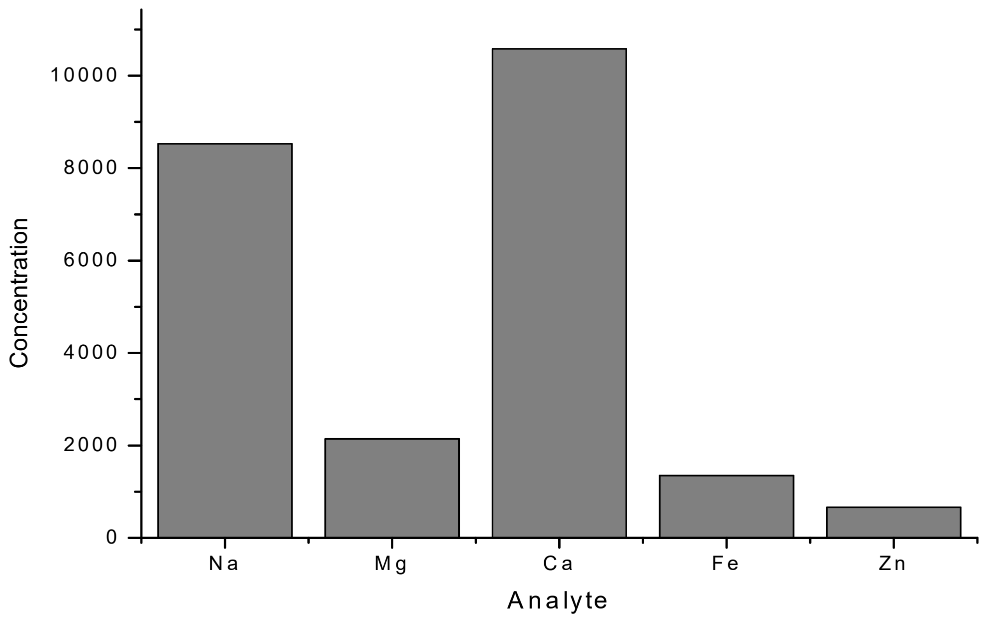


Figure 10. Concentrations of Na, Ca, Mg, Fe, and Zn in Victoria ouzo in µg/L.

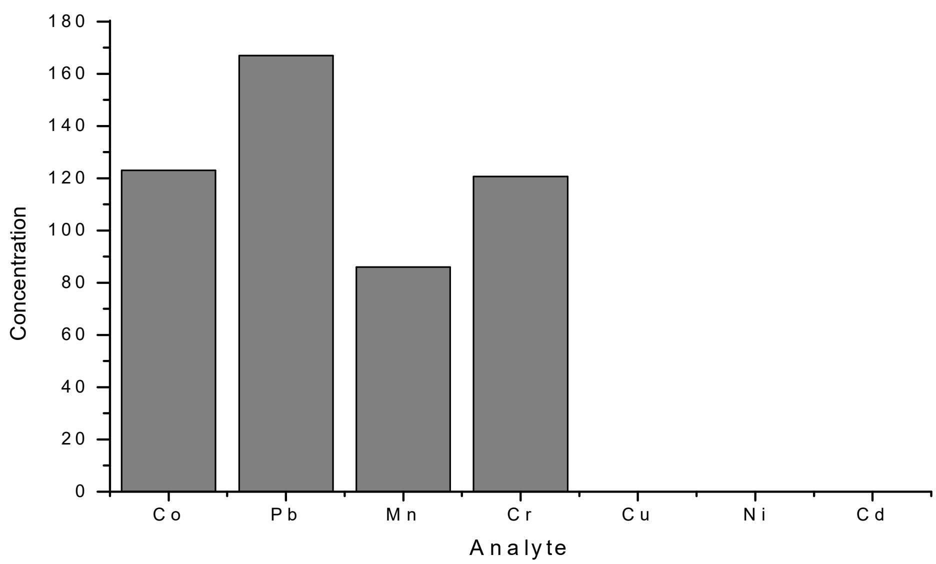


Figure 11. Concentrations of Co, Pb, Mn, Cr, Cu, Ni and Cd in Victoria ouzo in µg/L.

3.4.4. Concentration of metals in Liyu Addis ouzo

Liyu Addis ouzo is also one of the brands analyzed for its total metals concentration. Table 8 shows concentration of metals in Liyu Addis ouzo. The levels of metals are with the same trend with previous three brands. The results show that Na is with the highest levels 18194 ± 16 μg/L followed by Ca and Mg 11130 ± 7.5 μg/L and 10977 ± 20 μg/L respectively. From minor elements Fe, 2881 ± 10 μg/L is found to be with higher amount. Next is Zn and Mn with the values of 1368 ± 11 μg/L and 85 ± 0.2 μg/L respectively. It also contains Non essential metals in lesser quantity which is 323 ± 7 μg/L for Pb, 130 ± 2 μg/L for Co and 102 ±0.3 μg/L for Cr. In here also Cu (<2), Ni (<40) and Cd (<5) are below the detection limit of the instrument. The levels of metals can be ranked as

Na > Ca > Mg > Fe > Zn > Pb > Co > Cr > Mn and Cu, Ni and Cd are nil. Figure 12 and 13 shows concentration of metals analyzed in Liyu Addis ouzo.

Table 8 Levels of metals in Liyu Addis ouzo beverage

Metal	Na	Mg	Ca	Fe	Zn	Co
Concentration (μg/L)	18194 ±16	10977 ± 20	11130 ± 8	2881 ± 10	1368 ± 11	130 ± 2

Metal	Pb	Mn	Cr	Cu	Ni	Cd
Concentration (μg/L)	323 ± 7	85 ± 0.2	102 ± 0.3	-	-	-

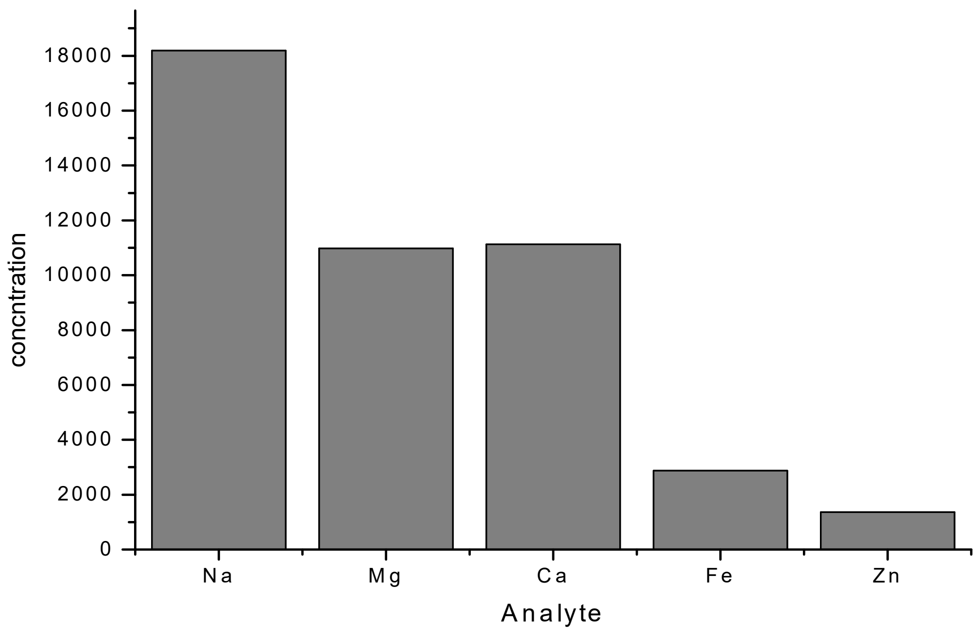


Figure 12. Concentrations of Na, Ca, Mg, Fe, and Zn in Liyu Addis ouzo in µg/L.

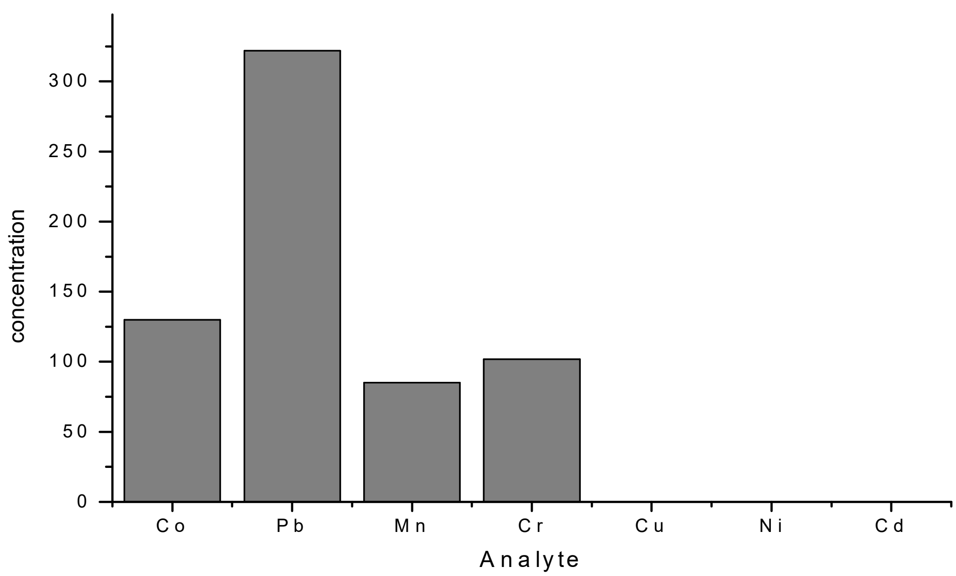


Figure 13. Concentrations of Co, Pb, Mn, Cr, Cu, Ni and Cd in Liyu Addis ouzo in µg/L.

3.4.5. Concentration of metals in Belezaf ouzo

The 5th chosen brand for the analysis of total concentration of metals is Belezaf ouzo and the levels of metal analyzed are given in Table 9. Similar to the previous four brands, the Belezaf ouzo also contain Na, 14002 ± 17 μg/L in higher amount. Ca and Mg, 12830 ± 23 μg/L and 1502 ± 20 μg/L, respectively are the next element found in higher amount. From the heavy metal Zn, 2215 ± 11 μg/L is found to be with the higher concentration followed by Fe, 1075 ±21 μg/L and Mn is the 3rd which is 225 ± 0.2 μg/L. Non-essential elements are also found in lesser amount like the previous brands. Pb 193 ± 3 μg/L is found to be with the higher concentration compared to the other non-essential metal and the 2nd and third are Co and Cr, 72 ± 0.6 μg/L and 71 ± 0.5 μg/L, respectively. In Belezaf ouzo also Cu (<2), Ni (<40) and Cd (<5) are found to be below detection limit of the instrument. The total levels of metal in Belezaf ouzo can be ranked as Na > Ca > Mg > Zn > Fe > Mn > Pb > Cr and Co. Co and Cr are found to with the same level in Belezaf ouzo. The total concentrations of metal analyzed in Belezaf ouzo are showed in Figure 14 and 15 graphically.

Table 9 Levels of metals in Belezaf ouzo beverage

Metal	Na	Mg	Ca	Fe	Zn	Co
Concentration (μg/L)	14002 ± 17	1502 ± 20	12830 ±23	1075 ± 21	2215 ± 11	72 ± 0.6

Metal	Pb	Mn	Cr	Cu	Ni	Cd
Concentration (μg/L)	193 ± 3	225 ± 0.2	71 ± 0.5	-	-	-

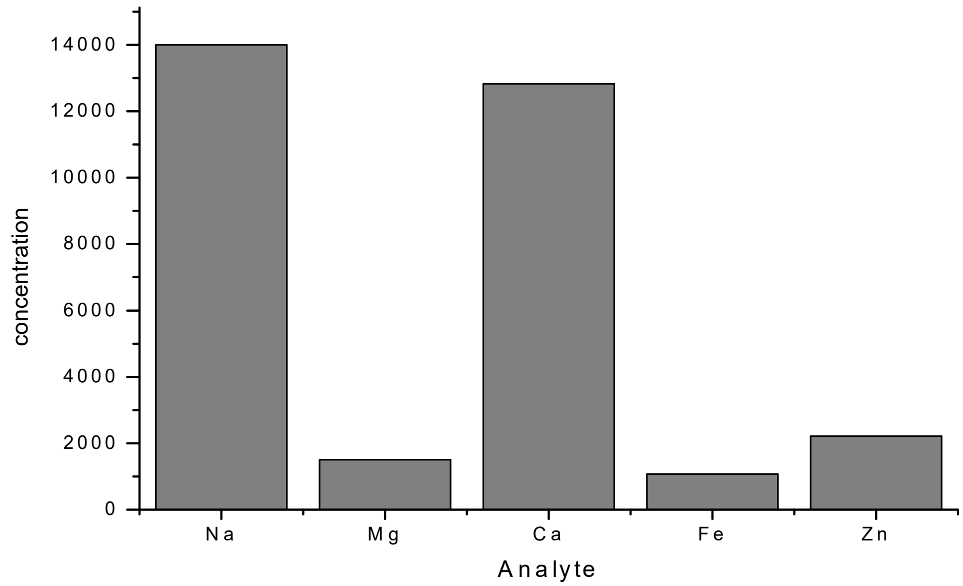


Figure 14. Concentrations of Na, Ca, Mg, Fe, and Zn in Belezaf ouzo in μg/L.

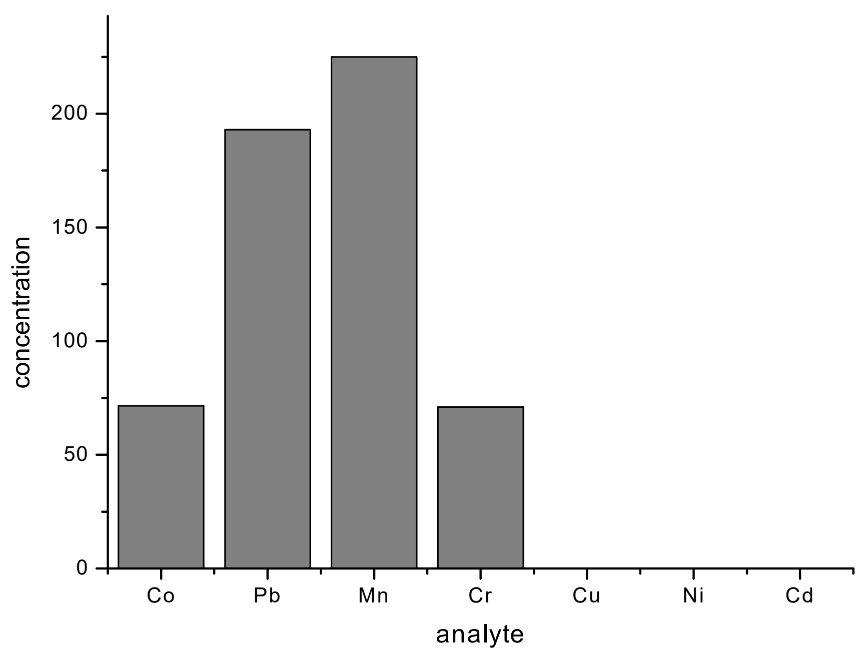


Figure 15. Concentrations of Co, Pb, Mn, Cr, Cu, Ni and Cd in Belezaf ouzo in μg/L.

3.5. Comparison of the concentration of metals in five Ethiopian local ouzo brands

Even though the trend of levels of essential and non-essential metals are the same; the total metal content of each brand is quite different, it lies in certain range. Table 10 gives the rank of each five brand from 1st to 5th for their total metal content and Table 12 shows the range of total metal concentration of each five brands analyzed.

Table 10 Levels of selected elements in Kokeb, National, Victoria, Liyu Addis and Belezaf ouzo drink.

metals	Concentration of metals in different ouzo brands (μg/L)					
	Kokeb	RSD	National	Victoria	Liyu Addis	Belezaf
Na	17711± 36	0.2	10079 ± 34	8529 ± 17	18194 ± 16.3	14002 ± 17
Ca	8834.4 ± 73	0.5	8330 ± 25	10583± 8.5	11130 ± 7.5	12830± 23
Mg	10208 ± 45	0.7	1345 ± 17	2140 ± 10	10977 ± 20	1502 ± 20
Fe	1010 ±68	0.9	942 ± 10	1346 ± 39	2881 ± 10	1075 ± 21
Zn	679 ±20	3	642 ± 20	660 ± 3.4	1368 ± 11	2215 ± 11
Mn	15.3 ± 0.2	0.83	130 ± 0.5	86 ± 0.2	85 ± 0.2	225 ± 0.2
Pb	507 ± 4	0.99	127 ± 5.6	167 ±1.5	323 ± 7	193 ± 3
Cr	54.4 ± 0.35	1.6	120 ± 0.3	121 ± 0.8	102 ± 0.3	71 ± 0.5
Co	-	-	-	123 ± 2.3	130 ± 2	72 ± 0.6
Cu	212 ± 20	9.4	-	-	-	-
Ni	-		-	-	-	-
Cd	-		-	-	-	-

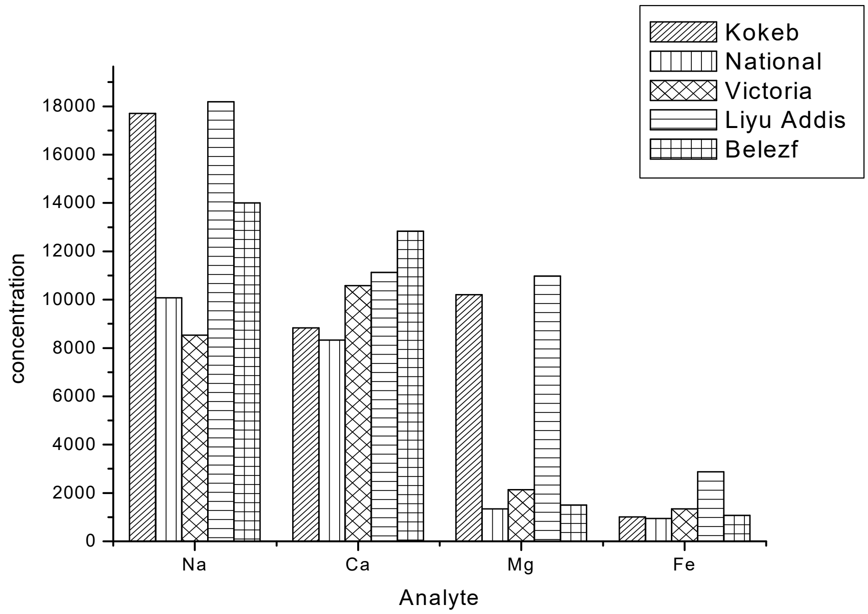


Figure 16. Levels (μg/L) of major metals in five ouzo brands.

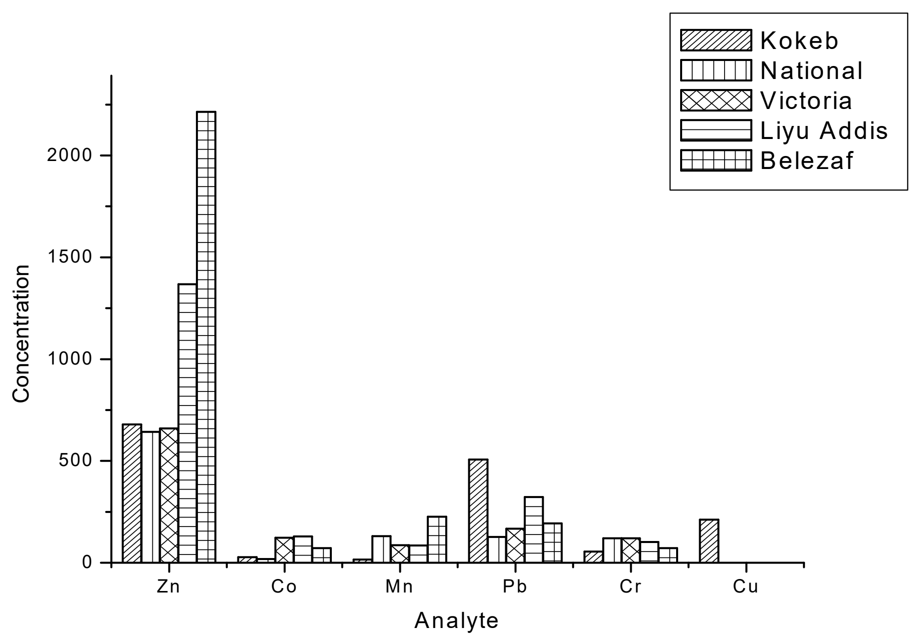


Figure 17. Levels (μg/L) of trace metals in five ouzo brands.

Table 11 Range of values of metal content (μg/l) of five different brands of ouzo beverage.

Metal	Range	Metal	Range
Na	18194 – 8529	Co	ND(<50) - 130
Mg	1345 – 10977	Pb	126.7 - 507
Ca	8330 – 12830	Mn	15 - 225
Fe	942 – 2881	Cr	54 - 121
Zn	642 – 2215	Cu	ND(<20) - 212

From Table 11 one can summarize that Liyu Addis, Belezaf, and Victoria ouzo alcoholic beverages are with the higher concentration of major elements and National ouzo beverage is with the least content of major element, lead and some trace metals. While Liyu Addis and Belezaf are relatively with higher concentration of trace metal and Kokeb and National are with less trace metallic content. Copper is detected only in Kokeb ouzo beverage; it is below the detection limit (<20) in other four brands.

Different reports indicate the metal content of different alcoholic beverage like wine, beer, and other distilled beverages. The levels of metals in Ethiopian ouzo are almost comparable with reported metal content of different alcoholic beverage like wine, beer, and other distilled beverages. Ouzo beverage can be compared with distilled spirits since the same category and method of preparation is similar. Table 12 shows levels of metal reported in different alcoholic beverage in comparison with present study.

Table 12. Comparison of levels of metals Ethiopian ouzo with that in beer, wine, and distilled spirit from different countries.

Alcoholic beverage	Concentration of major metals (µg/l) in alcoholic beverage						
	Na	Ca	Mg	Fe	Mn	Zn	Reference
Beer	NR	5000-10000	2000-5000	NR	110-348	52.7-166	[19, 33]
Wine	8000-24200	27700-68100	21000-75100	700-7300	500-4400	50-1800	[12,22]
Distilled spirits	17800-161800	8000-14800	1410-4140	350-2030	NR	NR	[23]
Ethiopian ouzo	8529–18194	8330 – 12830	1345 – 10977	942 – 2881	15 - 225	642 – 2215	Current study

∗ Results are the average of three reading of triplet analysis.

NR = not reported.

ND = not detected

Alcoholic beverage	Concentration of trace metals (µg/L) in alcoholic beverage						
	Pb	Co	Cr	Cu	Ni	Cd	Reference
Beer	65-105	NR	NR	38-144	NR	ND	[19, 33]
Wine	50-500	ND-40	10-260	10-1650	ND-130	ND-30	[14,17]
Distilled spirits	32-548	NR	85-183	ND- 105	ND-22	ND	[18, 21]
Ethiopian ouzo	127- 507	ND(<50) - 130	54 - 121	ND- 212	ND	ND	Current study

3.6. Statistical analysis of data (ANOVA)

Different statistical methods have been used to check whether there is a difference in results of analysis or not. Analysis of variance (ANOVA) is one of these methods used to identify the source of variability from one or more potential sources [69]. Analysis of variance (ANOVA) is used to perform comparison of the means of a number of replicates of experiment performed. For this study, the significance of variation between the samples has been studied using one-way ANOVA. Such calculation can be made using detail calculations following the formula. It can also be made on a computer using Excel, Origin and Minitab. For the present study Minitab was used to calculate the presence or absence of significant difference in mean concentration of each metal between five brands of ouzo namely Kokeb, National, Liyu Addis, Victoria and Belezaf for each metal.

For each of the analysis six means, three for each ouzo sample were used at 95% confidence level or 0.05 significance level. There is no significant difference in the levels each particular metal in the five ouzo brands for $p > 0.05$. Significant difference is observed for $p < 0.05$. There is significant difference ($p < 0.05$) in levels of sodium metal between Kokeb and National ouzo ($p = 0.00368$), Kokeb and Victoria, National and Liyu Addis, National and Belezaf, Victoria and Liyu Addis, Victoria and Belezaf and Liyu Addis and Belezaf, But there is no significant difference between ($p > 0.05$) Kokeb and Liyu Addis, Kokeb and Belezaf ($p = 0.05774$), National and Victoria.

For calcium significant difference is observed only between Kokeb and Belezaf, National and Victoria, National and Liyu Addis and National and Belezaf at $p < 0.05$. There is no significant difference between the rests of ouzo alcoholic beverage. In the case of magnesium there is significant difference $p < 0.05$ between Kokeb and National, Kokeb and Victoria, Kokeb and Belezaf, National and Victoria, National and Liyu Addis, Victoria and Liyu Addis, Victoria and Liyu Addis Victoria and Belezaf and Liyu Addis and Belezaf. The level of magnesium is not significantly different ($p < 0.05$) in two ouzo brands Kokeb and Belezaf and National and Belezaf.

In a similar manner it is possible to compare the differences of the levels of minor metals in each ouzo brands. In the case of Fe notable difference in levels are observed between Kokeb and Victoria, Kokeb and Liyu Addis, National and Victoria, National and Liyu Addis, Victoria and Liyu Addis, Victoria and Belezaf, Liyu Addis and Liyu Addis at $p < 0.05$. There is no notable difference between Kokeb and National, Kokeb and Belezaf and National and Belezaf $p > 0.05$. Remarkable difference is observed in Kokeb and Liyu Addis, Kokeb and Belezaf, National and Liyu Addis, Victoria and Liyu Addis, Victoria and Belezaf and Liyu Addis and Belezaf and no significant difference between the rests of the brands in the case of zinc. Except for Liyu Addis and Victoria and Victoria and Belezaf there is significant difference in the levels of cobalt between each ouzo brands at $p < 0.05$. In the case of lead, similar to previous case the result shows that notable difference is observed between each brands except for National and Victoria, National and Belezaf and Victoria and Belezaf in which there is no considerable difference in levels of lead at $p < 0.05$.

For Mn and Cr the difference in the means are significantly different ($p < 0.05$) between Kokeb and National, Kokeb and Victoria, Kokeb and Liyu Addis, National and Belezaf, Victoria and Liyu Addis, Victoria and Liyu Addis and Liyu Addis and Belezaf and insignificant ($p > 0.05$) in between Kokeb and Belezaf, National and Victoria and National and Liyu Addis pairs.

The analysis of variance reveals that there is significant variation in levels of elements between each brands of ouzo alcoholic beverage. The differences might be due to the type and concentration of flavoring used and environmental condition in which the plant grow through. Not only this, the type of water used for dilution will determine the levels of elements in Ethiopian ouzo alcoholic beverage.

4. Conclusion

Ethiopian ouzo is common alcoholic beverage consumed in different parts of Ethiopia and all over the world. In this study the levels of selected major and minor elements ((Na, Ca, Mg, Fe, Zn, Mn, Co, Cr, Pb, Cu, Ni and Cd) for different ouzo brands (Kokeb, National, Victoria, Liyu Addis and Belezaf) were determined.

The optimized wet digestion method for the analysis of ouzo was found to be efficient for the majority of the minerals and it was evaluated through the recovery experiment and a good percentage recovery was obtained (100 ± 5) for the majority of the minerals identified. The results showed that the levels of metals in all five brands Ethiopian ouzo brands are in appropriate concentration. Generally the levels of metals are higher in Liyu Addis compared to the rest. The ANOVA results suggest that there were significant variation in the level of some elements in different ouzo brands, which result from different factors. This is due to different precaution taken during processing, fermentation and storage conditions, amount of flavoring added and environment through which flavoring grow and extracted. In all five ouzo brands the levels of major elements are in higher concentration, minor elements in medium quantity and non-essential elements Ni and Cd are not detected. Some trace metals (Pb, Mn, Cr and Co) are also found in lower concentration. Cu detected only in Kokeb ouzo beverage and it was below detection limit (<2) in the rest of ouzo brands. The levels (μg/L) of metals can be arranged in increasing order as Na (18194 - 8529) > Ca (8330 - 12830) > Mg (1345 – 10977) > Fe (942 – 2881) > Zn (642 - 2215) > Pb (126.7 – 507) > Mn (15 - 225) > Cr (54 - 121) > Co (ND <50 - 130) > Cu (ND <20 - 212) in increasing order. Except for Zn and Co, the results obtained in this study are comparable with reported literature values analyzed from other parts of the country for other alcoholic beverage like beer, wine and other distillate.

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Declaration

I the undersigned confirm that the results reported in this work were obtained by research carried by me under the supervision of my advisor in the Faculty of Science, Department of Chemistry, Addis Ababa University in the academic year 2007/2008.

Name: Dereje Bekele Abebe Signature ____________________________

This project has been submitted for examination with my approval as a university advisor.

Advisor: Prof. B. S. Chandravanshi

Signature________________________

Place and date of submission: School of Graduate Studies

Addis Ababa University

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