ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Determination of Selected Trace Metals and Physicochemical Parameters of Commercially available Honey in Ethiopia

By:

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July 2009

Determination of Selected Trace Metals and Physicochemical Parameters of Commercially available Honey in Ethiopia.

Graduate Project

(Chem. 774)

Submitted To:

Department of Chemistry

In Partial fulfillment of requirements for the Degree of Master of Science in Chemistry

By:

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July 2009

Acknowledgements

I would like to express my deepest gratitude and thanks to my advisors, Prof. B. S. Chandravanshi and Dr. Ghirma Moges for their unreserved cooperation, suggestions, supervisions and remarks, appreciable encouragement, fatherly hood consultancy and taking their time to read and correct my project paper.

I am very grateful to Addis Ababa University for providing me sponsorship and for its provision of all resources necessary to accomplish my study from the very beginning to the end.

I am also thankful to staff member of Chemistry Department, Dr. Ashebir Fiseha, Head of Department of Chemistry, Addis Ababa University and Dr. Yonas Chebude, for their kindly cooperation in facilitating conditions and allowing resources throughout my study.

I would like to extend my thanks to, Ato Hailegebriel Mengesha, W/t Amakelech Belete and W/o Woinshet Gebeyehu for their cooperation in laboratory work.

Ato Sahilemicheal Deme also deserves to be acknowledged for his cooperation in providing laboratory apparatus and chemicals on time.

Finally, I am grateful to my family for their support, motivation and encouragement during my study.

Weldegebriel Yohannes

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Lists of Acronyms and Abbreviations

EC	Electrical Conductivity
WHO	World Health Organization
FAO	Food and Agriculture Organization
ANOVA	Analysis of Variation
% RSD	Percentage Relative Standard Deviation
SD	Standard Deviation
ND	Not Detected
NDR	Not Determined
EU	European Union
MTL	Maximum Tolerable limit
FAAS	Flame Atomic Absorption Spectrometry
AOAC	Association of Official Analytical Chemists
ES	Ethiopian Standard

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Abstract

Trace mineral content and some quality parameters (physicochemical properties) such as pH, electrical conductivity and ash content of honey were determined using FAAS, pH- meter, conductivity meter and furnace, respectively. Fe was found in highest amount with mean concentration ranging from 5.37 to 12.43 µg/g followed by Ni with mean concentration range of 0.8 to 4.46 µg/g, Cr (1.20-4.33 µg/g), Zn (1.92-4.22 µg/g), Co (0.60-1.17 µg/g), Cd (ND-0.69 µg/g), Mn (0.16-0.885 µg/g) and Cu (0.09-0.4676 µg/g). The non-essential metal Pb was not detected.

Similarly, the ranges of physicochemical properties analyzed could be summarized as: pH 4.11-4.33, ash content 0.17-0.46 % and electrical conductivity of 0.10-0.29.

The metals content and the physicochemical properties investigated in honey samples were found within the ranges established by national and international standards.

The quality of some samples of honey was evaluated from the point of view of physicochemical properties and content of trace metals.

The optimized wet digestion method for honey analysis was found efficient for the metals analyzed and it was validated through the recovery experiment and a good percentage recovery was obtained (93-104%).

Key words: **Honey, Essential trace metals, Non-essential metals, physicochemical properties, FAAS.**

1. Introduction

1.1. Definition

Honey is a natural, sweet, viscous liquid that honey bees *Apis mellifera* produce from nectars of blossoms, from plant secretions or from excretions of plant sucking insects on the living parts of plants [1-7]. Honeybees collect this material, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature [4-6, 8]. Freshly extracted honey is a viscous liquid, with a greater density (1.5 g/cm^3) than water (1 g/cm^3 at 4 ºC); having a strong hygroscopic character, relatively low heat of conductivity, low surface tension and various colors [1].

Honey is an easily digestible foodstuff that contains a range of nutritionally important compounds [9, 10]. The major components of honey include various saccharides, water, amino acids, proteins, vitamins, and unstable compounds such as enzymes [3, 4, 7, 9]. In addition to these, honey contains inorganic substances, including trace elements essential for vital processes. The composition and properties of honey depend on the sort, location, environment, collection time, climatic conditions [11 - 15].

The composition of nectar and honeydew honeys is different. Light nectar honeys have a lower content of components than darker honeys [11, 12, 16]. In general, higher antioxidant content was found in darker honeys and in honeys with higher water content [1, 16].

Food safety is a major public concern worldwide. During the last decades, the increasing demand for food safety has stimulated research regarding the risk associated with consumption of foodstuffs contaminated by pesticides, heavy metals and/or toxins [17].

The current international honey market trend, regarding quality is every day more demanding. Hence, it is necessary to promote all feasible activities in order to produce residue free honey [18]. No food ingredient additives, such as: color, vitamins and

minerals or preservatives to honey are allowed and also it shall not have any objectionable matter, flavour, aroma, or taint absorbed from foreign matter during its processing and storage [3, 4, 7, 19]. However, honey can be contaminated involuntarily from the environment and from beekeeping practice [20].

1.2. Origin and distribution of honey

As the only available natural sweetener honey was an important food for Homo sapiens from his very beginnings. Indeed, the relation between bees and man started as early as Stone Age [21]. From ancient times, honey was not only used as a natural sweetener but also as a healing agent [16]. In most ancient cultures honey has been used for both nutritional and medical purposes [2, 21].

It was used by the ancient Greek sand Sumerians. In ancient Egypt it was used as a wound treatment, mixed with grease and fibre and for gut conditions. Hippocrates recommended honey and vinegar for pain, water and honey for thirst and a mixture of honey, water and other substances to treat acute fevers, as well as recommending its use to treat ulcers.

The belief that honey is a nutrient, a drug and an ointment has been carried into our days. For a long time in human history it was an important carbohydrate source and the only largely available sweetener until industrial sugar production began to replace it after 1800. An alternative medicine branch, called apitherapy, has developed in recent years, offering treatments based on honey and the other bee products against many diseases.

Russian soldiers in World War 1 used it, apparently successfully, for wound healing purposes. Honey, lime leaves and palm kernel are traditional medicines for wound healing in Ghana and among the Bambara of Mali; honey is a traditional treatment for measles, both via the oral route and as an eye ointment [2, 22].

At present the annual world honey production is about 1.2 million tons, which is less than 1% of the total sugar production. The consumption of honey differs strongly from country to country. The major honey exporting countries China and Argentina have small annual consumption rates of 0.1 to 0.2 kg per capita. Honey consumption is higher in developed countries, where the home production does not always cover the market demand. In the European Union, which is both a major honey importer and producer, the annual consumption per capita varies from medium (0.3-0.4 kg) in Italy, France, Great Britain, Denmark and Portugal to high (1-1.8 kg) in Germany, Austria, Switzerland, Portugal, Hungary and Greece, while in countries such as USA, Canada and Australia the average per capita consumption is 0.6 to 0.8 kg/year [21].

Table1. Countries listed below accounted for almost 65% of global honey out put in 2005 [23]

No	Country	Amount produced (in thousand metric tones)	Global honey production (%)
1	China,	298	21.5
2	Turkey	82.3	5.9
3	Argentina	80	5.8
4	United States	79.2	5.1
5	Ukraine	71.5	5.1
6	Russia	52.1	3.8
7	India	52	3.7
8	Mexico	50.6	3.6
9	Ethiopia	39	2.8
10	Spain	37	2.7

1.3. Honey Production in Ethiopia

Of all countries in the world probably no country has a longer tradition of beekeeping than Ethiopia. Already the hieroglyphs of the ancient Egyptians give a hint, that this country has been a source for honey and beeswax ever since. The dissemination of Christianity moreover strengthened the beekeeping system because of its demand for wax for religious ceremonies. Today Ethiopia owns with about 10 millions of bee colonies, the largest bee population in Africa. Ethiopia is the largest honey producer in Africa and the ninth largest honey producer all over the world. The total honey production of Ethiopia is estimated up to be 43 000 tons, and only a small portion of this is marketed. Beside poor marketing conditions the main reason is that about 80% of the total Ethiopian honey production goes into the local "Tej" preparation, (*honey mead),*a national drink consumed in large quantities [24].

More than 95% of the honey in Ethiopia is produced through traditional hives. In recent years few honey processing enterprises have appeared in the market, involved in the production of cream and table honey. Mostly, the enterprises collect honey from groups of outgrower farmers in Southern Ethiopia (Keffa region, Agaro Area), Gojam, Gonder, Wollo and Tigray. Those enterprises plan to export abroad including the European Union and the Middle East markets.

1.4. HONEY PROCESSING

Heating

Heating is the basic processing treatment of bee honey. The principal objective of heating is to change the state of the product from solid to liquid. Additionally, when properly combined with filtration, it prolongs the re-crystallization of honey. The effect of heating and filtration on honey re-crystallization has been generally recognized for all long time. Based on those two treatments, a number of methods were developed which are devised

to maximize the duration of the liquid state of the product. Another method recommends heating honey up to 77 °C for 5 min. and, subsequently, filtering it carefully and cooling down rapidly to room temperature. There is yet another approach to honey processing that makes honey stay liquid for a long time, a method that additionally makes use of small quantities of water being added at the initial heating stage to facilitate the dissolution of crystals [25, 26].

The use of excessive heat in honey processing for liquefaction or pasteurization, however, has adverse effects on honey quality, i.e. loss of volatile compounds, accumulation of HMF and reduction of invertase and diastase activities. However, it should be noted that improper storage of honey leads also to similar changes of HMF and enzyme activity.

Honey filtration

Honey should not be strained with a mesh size smaller than 0.2 m in order to prevent pollen removal. On the other hand, the recently revised Codex Alimentarius Honey Standard (Codex Alimentarius Commission 2001) and EU Directive relating to honey [6, 27] allow a removal of pollen if it is unavoidable for the removal of foreign matter. Such honey should be labeled as “filtered”. Since microscopical pollen analysis is still the most important tool for the determination of botanical and geographical origin of honey, any removal of pollen by filtration will make authenticity routine testing much more difficult, if not impossible.

Fermentation

Harvesting of honey with high moisture content, or subsequent addition of water can result in honey fermentation and spoilage. Honey spoilage can be first tested by a microscopic yeast count .This test on its own does not yield conclusive results, as counted yeast could be in an inactive status not taking part in the fermentation process.

Determination of the fermentation products is more reliable, i.e. by determining the glycerol or ethanol content [19].

1.5. Nutritional benefits of honey

Many health-promoting and curative properties attributed to honey are the basis for some traditional folk medicine treatments throughout the world today [16]. There are many reports of the traditional medicinal use of honey in a large number of cultures. It has been used in a wide range of conditions, including skin, eye, respiratory and gastrointestinal illnesses [2, 21, 22].

If honey is consumed at higher doses of 50 to 80 g per intake, it has a variety of positive nutritional, healing, and prophylactic properties [16, 21, 29, 30].

Bee honey can be a good source of major and trace elements needed by humans. The mean content of mineral substances in honey has been calculated to be 0.17%**,** although this can vary within a wide range [8, 31].

In order to have a beneficial effect, honey must be free of any contaminating agents. Any heavy metals present in honey above the admitted levels by pollution standards, are threats to human body through the possible negative effect of the contaminants [2, 29, 32, 33].

As a widely consumed food, honey has a very long history of safe use. Liquid honey does not spoil because of its high sugar concentration and low moisture content, which kills most bacteria by plasmolysis and impedes the development of airborne yeasts. The moisture content of natural raw honey varies from 17.5 to 21 %. As long as the moisture content remains below 18%, virtually no microorganisms can successfully multiply in honey [3, 34].

The smaller amount of moisture in honey is not the only explanation for its antimicrobial activity. Some studies have studied sugar syrups of the same water activity as honey and found them to be less effective than honey at inhibiting microbial growth [2, 16].
When applied topically to wounds, osmosis would be expected to draw water from the wound into the honey, helping to dry the infected tissue and reduce bacterial growth [2, 35].

Honey has been demonstrated in many studies to have antibacterial effects, attributed to different factors: its high osmolarity (due to its high sugar content), low pH, hydrogen peroxide content and content of other uncharacterized compounds.

Honey is mildly acidic, with a pH between 3.2 and 4.5. Gluconic acid is formed in honey when bees secrete the enzyme glucose oxidase, which catalyses the oxidation of glucose to gluconic acid. The low pH alone is inhibitory to many pathogenic bacteria and, in topical applications at least, could be sufficient to exert an inhibitory effect [2, 5, 16].

Honey has been also claimed to have therapeutic properties applied as ointments for the treatment of minor burns, cuts and skin infections, in the treatment of digestive, respiratory, cardiac and rheumatic disorders. It may be suitable for use in oral rehydration products [2, 4, 22, 29].

1.6. Chemical Composition of honey

Honey contains about 181 substances [36]. The various chemical components of honey include: carbohydrates that comprise the major portion of honey, small amounts of proteins, minerals, vitamins, aroma compounds, organic acids and poly phenols[2, 6, 13, 16, 21,].

1.6.1. Carbohydrates

The sugars fructose (approximately 38% w/w) and glucose (~31%) are the two main sugars present in honey, with lesser amounts of sucrose (~1%). Additionally, about 25 different oligosacharides have been detected [11, 21].

In the process of digestion after honey intake the principal carbohydrates fructose and glucose are quickly transported into the blood and can be utilized for energy requirements by the human body. A daily dose of 20 g honey will cover about 3% of the required daily energy [11]. In other words it is a more concentrated source of energy than other common sweeteners. Furthermore, besides its high nutritional value (330 kcal/100 g) its carbohydrates are absorbed rapidly on consumption [5].

1.6.2. Proteins

Honey contains roughly 0.5% proteins, mainly enzymes and free amino acids. The contribution of that fraction to human protein intake is marginal [11]. Enzymes are the most important and also the most interesting honey components. They are accountable for the conversion of nectar and honeydew to honey [37]. The three main honey enzymes are diastase (amylase), decomposing starch orglycogen into smaller sugar units, invertase (sucrase, α-glucosidase), decomposing sucrose into fructose and glucose, as well as glucose oxidase, producing hydrogen peroxide and gluconic acid from glucose. Further more, honey has eighteen free amino acids, carboxylic acid group, of which most abundant is proline [1, 2,4,11, 16, 38].

1.6.3. Vitamins, minerals and trace compounds

Honey contains trace amounts of the B vitamins: riboflavin, niacin, folic acid, pantothenic acid and vitamin B_6 and ascorbic acid [1, 7]. It also contains varying amounts of mineral substances ranging from 0.02 to 1.03g/100g, minerals such as: calcium, iron, zinc, potassium, phosphorus, magnesium, selenium, chromium, manganese, etc. and

organic acids such as acetic, butyric, citric, succinic, lactic, malic, and gluconic acid and a number of aromatic acids [1].

The chemical composition of honey is influenced by many factors. It varies depending on plant source (botanical origin of the nectar) visited by bees, season, environmental conditions and production methods but the main constituents are the same in all honeys [2, 8, 15, 21, 22, 39]. Storage conditions may also influence final composition, with the proportion of disaccharides increasing over time [2, 40].

1.7. Sources of residues and contaminats in honey

Honey has two sources of residues pesticides and veterinary drugs as well as contaminants **(**microbial and heavy metal ions). The primary sources of both residues contaminats include pollen, dust, air, soil and nectar; secondary sources are those arising from honey manipulation by people, they include cross-contamination by food-handlers, containers and packaging, transport, storage facilities, honey extractors and processing machines. Primary sources of honey contamination are very difficult to control. Conversely, secondary sources of residues and contaminants in honey can be controlled by good apicultural and manufacturing practices.

Detectable pesticides and veterinary drugs arise from the poor agricultural practices where residues in the nectars of flowers will be collected by the bees. No application of pesticides and drugs is a guarantee to arrest residues. There are two ways to avoid residues in honey from the sources (i) no application of these substances in the area close to the apiary (organic practice); (ii) good apicultural and apicultural practices in the uses of these substances

It is important to take account of the type of equipment used to produce honey as well as the quality of the equipment used to store honey after harvesting as possible sources of honey contamination with heavy metals. For example contact with stainless steel surfaces during harvesting, processing and/or preparation of honey for the market, can

generate high Cr content, due to the corrosive effect of honey acidity. Likewise, storing honey in galvanized containers can be a source of Zn contamination [30]. However, variation in trace element content in different honey types is primarily due to botanical origin rather than geographical and environmental exposition [41].

The concentrations of inorganic species present in honey vary according to the resources in the soil [20]. Plants absorb elements from the earth and deliver them to the nectar, which is a major resource used by bees to make honey. Honey will vary in mineral content not only according to the resources in the soil where its evolution starts, but also according to the kind of plants from which the bees took nectar [20, 31].

1.8. Essential and Toxic Metals

As food stuff used for healing purposes, honey must be free of objectionable contents. It should contain only small amounts of trace metals [30]. Any heavy metals present in honey above the admitted levels by pollution standards, are threats to human body through the possible negative effect of the contaminants [29].

Heavy metals in foods and beverages are classified into two based upon their essential and toxic nature. For example Fe, Zn, Cu, Mn, Cr and Co are described as essential trace elements for humans. Apart from these trace elements which are indispensable for the human body, there are other groups of trace elements such as Pb, Cd, As and Hg which are toxic even in minute quantities [7, 8, 12, 30, 31, 42 - 44].

Trace elements may play an important role in a number of biochemical processes; they ensure the natural development of physiological reactions, take part in metabolism and impact general metabolism, circulatory systems and influence the reproduction as catalysts of various biochemical reactions. Trace elements are the constitutive parts of the structures of different active bio-compounds: zinc, copper and manganese are found in enzymes, cobalt in vitamins and hormones, copper and iron in respiratory enzymes [8, 13, 30, 44].

Lead, cadmium arsenic and mercury are potentially toxic within specific limiting values. Excessive content of these metals in food is associated with a number of diseases, especially of the cardiovascular, renal, nervous and skeletal systems. These heavy metals are also implicated in carcinogenesis, mutagenesis and soon [13, 17, 30, 44]. Therefore, honey shall be free from heavy metals in amounts which may represent a hazard to human health [6].

1.9. Roles played by essential and non essential metals

General roles played by the trace heavy metals: Fe, Zn, Mn, Cu, Co, Cr, Pb, Ni and Cd are presented in the following section.

1.9.1. NICKEL

Nickel is now quite firmly established as an essential nutrient [42] and its compounds are generally recognized as safe when used as a direct ingredient in human food. Little is known about the actual chemical forms of nickel in various foods or whether dietary nickel has distinct “organic” forms with enhanced bioavailability analogous to those of iron and chromium. Although a number of cellular effects of nickel have been documented, a deficiency state in humans has not been described. Research showed that nickel was to be found in blood and tissues at quite consistent levels associated with DNA and RNA in amounts that suggest physiological significance [45].

Nickel is frequently responsible for allergic skin reactions and has been reported to be one of the most common causes of allergic contact dermatitis, as reflected by positive dermal patch tests [44, 45].

1.9.2. Zinc

Zinc is a multifunctional nutrient involved in glucose and lipid metabolism, hormone

function, and wound healing, and it is also essential in proper hair growth [46]. But excess intake of it results in gastrointestinal distress and diarrhea [8]. Zinc is a component of enzymes or a functional cofactor of a large number of enzymes. It is essential to carbohydrate metabolism; protein synthesis [47].

Zinc is an essential trace element that must be supplied in the diet of human beings so that growth and health can be maintained. It is necessary for protein synthesis and the metabolism of vitamin A. It helps the healing process of internal and external wounds, decreases cholesterol deposits and promotes mental awareness. A deficiency can cause loss of appetite, growth retardation and immunological abnormalities [48, 49].

1.9.3. Copper

Copper plays important roles in normal carbohydrate and lipid metabolisms. Copper and iron are essential to life because they play major roles in blood building and the functioning of critical enzyme systems [46].

Copper is essential for good health but very high intake can cause adverse health problems, such as liver and kidney damage [8].

1.9.4. Iron

In animals, Iron is a constituent of hemoglobin. Body iron content is regulated by the amount absorbed. The absorption is influenced by body stores and by the amount and type of iron in ingested foods.

It is a vital component of many enzymes; it can promote resistance to disease and prevent fatigue. A deficiency can cause anemia, resulting in impaired concentration, reduced physical performance and work capacity, and decreased immune function. Ascorbic acid is necessary for the proper assimilation of iron. There are no reported cases of toxicity

from foods but iron poisoning may occur from ingesting large amounts of medicinal iron supplements [48, 49].

1.9.5. Manganese

Manganese is an essential component of numerous enzymes involved in bone formation and in the metabolism of amino acids, lipids, and carbohydrates. Its deficiency has been reported in animals but rarely in human [48, 50].

A deficiency can cause poor reproductive performance, growth retardation, abnormal formation of bone and cartilage, and an impaired glucose tolerance [48].

1.9.6. Cobalt

It is a part of vitamins B_{12}, an essential vitamin in animal nutrition. Pernicious anemia can result from cobalt deficiency, for which Vitamin B_{12} is a well-known treatment, being organically complexed with cobalt.

1.9.7. Chromium

Chromium is another micronutrient for animals. Trivalent chromium is required for maintaining normal glucose metabolism. Evidence shows that chromium improves glucose tolerance. Diabetes and coronary heart disease are associated with low chromium concentrations in human tissue. The chemical forms of chromium in foods are not known with certainty, but the bioavailability of chromium compounds has been found to be high in brewer's yeast, shellfish, whole wheat bread and mushrooms [51].

1.9.8. Cadmium

Because of its toxicity, presents a major problem for foodstuffs. Contamination through fertilizers becomes an increasing problem. Cadmium is concentrated particularly in the

kidneys, the liver, the blood forming organs and the lungs. It most frequently results in kidney damage (necrotic protein precipitation) and metabolic anomalies caused by enzyme inhibitions.

The decisive point is whether absorption of the existing cadmium actually takes place. This is, firstly, dependent upon the composition of the diet as a whole and, secondly, on the bio-availability of the cadmium compound present. No connection with cancerous disorders has been found [4, 44].

1.9.9. Lead

Lead which is known to be xenobiotic has no beneficial effects in human physiology but has detrimental effect to the physiologies of humans and other living organisms. It is a typical cumulative poison [45, 46].

Lead can trigger both acute and chronic symptoms of poisoning. Acute intoxications only occur through the consumption of relatively large single doses of soluble lead salts. Chronic intoxications can arise through the regular consumption of foodstuffs only slightly contaminated with lead. Lead is a typical cumulative poison.

Basically, as a result of their comparatively high affinity for proteins, the lead ions consumed bond with the hemoglobin and the plasma protein of the blood. This leads to inhibition of the synthesis of red blood cells and thus of the vital transport of oxygen. If the bonding capacity here is exceeded, lead passes into the bone-marrow, liver and kidneys. Such intoxication leads to:

I. Encephalopathy in the central nervous system (CNS);
II. Disturbances in kidney and liver functions progressing as far as necrosis;
III. Damage to the reproductive organs;
IV. Anemia and many metabolic deficiency symptoms.

Particularly dangerous to all forms of life are the organic lead compounds. They cause injuries to mental development such as reduction of intelligence and growth disturbances [4, 44].

1.10. Physicochemical properties of honey

Honey specifications (e.g. Ethiopian Standard, ES: 1202:2005 and the Revised Codex Standard) [6, 34] require physicochemical criteria for honey characterization –namely, water content, ash, pH, electrical conductivity, HMF, glucose, fructose, sucrose as well as diastase activity. Reported works in the published scientific journals also reflect the importance of these honey specifications [8, 15, 18, 34].

Chemical, physicochemical and palynological analyses have been used to characterize honey samples in order to distinguish their source type (honeydew or nectar) or floral origin or to control their quality [39].

1.10.1. Colour

The colours of honey form a continuous range from nearly water white to dark amber [6]. The colour of honey is related to its mineral content and is characteristic of its floral source. Light coloured honey typically has a mild flavor, while dark colored honey is usually stronger in flavor [52].

Figure1. The different colours of honey

The contents of essential trace elements also participate in the nutritional value of honey and it is one of the factors affecting the colour of honey [37].

1.10.2. Moisture content

The amount of water in honey is a function of many factors involved in ripening, including on the botanical origin of the sample, weather conditions, the original moisture of the nectar, harvest season, the conditions of storage and the degree of maturity [8].

Honey has moisture content with a range of between 15.1 and 21.0 %. As long as the moisture content remains below 18 %, virtually no microorganisms can successfully multiply in honey [3, 5, 34]. Anomalous values may be an index of adulterations [5].

Due to high abundance and low cost of water it is the most frequently added substance to various foods in an attempt of economic fraud [8, 53].

The water content is the most important measurand related to honey quality, especially concerning the risk of spoilage due to fermentation [8]. And hence it is important parameter in honey preservation [36].

Generally honeydew honeys have lower water content than blossom honeys. However, water content can be artificially altered during honey processing and is therefore not a reliable indicator for the botanical origin [53].

1.10.3. The pH value of Honey

All honeys are acidic with a pH-value generally lying between 3.5 and 5.5, due to the presence of organic acids that contribute to honey flavour and stability against microbial spoilage [53].

The pH is indeed a useful index of possible microbial contamination and adulteration. The pH of adulterated honey samples is higher than that of pure samples [3]. And has high relevance during the extraction and storage of honey because it is related to the stability and the shelf life of the product. Most bacteria and moulds grow in a neutral and mildly alkaline environment respectively.

1.10.4. Electrical conductivity

Electrical conductivity depends predominantly on the mineral content of honey [54]. This mesurand was recently included in the international standards replacing the determination of ash content [53, 54].

Electrical conductivity can be determined with an inexpensive conductometer and was found to be the most important variable for the classification of unifloral honeys. The range of electrical conductivity in honey lies between 0.06 and 2.17 $mscm^{-1}$. Honeydew contains considerably higher amounts of minerals compared to blossom honeys.

Generally honeydew honeys have an electrical conductivity higher than 0.8 $mscm^{-1}$ blends between blossom and honeydew honeys have conductivity values between 0.51 and 0.79 $mscm^{-1}$ and pure floral honeys exhibit conductivity values between 0.15 and 0.50 $mscm^{-1}$. A reliable determination of the botanical origin, however, can not be based on electrical conductivity only [53].

1.10.5. Ash content

The ash content in honey is generally low and influenced by the chemical composition of nectar that varies according to the different botanical sources involved in honey formation. It can vary between 0.02 and 1.0 % and the maximum limit allowed by legislation for honey from floral sources is 0.6 % [6, 27]. Normally, however, ash contents between 0.1 and 0.3 % are found for floral honeys. Very high mineral contents about 1.0 % are actually encountered only in honeydew honey and ash content is often used to identify this kind of honey [55].

1.11. Honey as bio-indicator

Analysis of honey for trace elements content is necessary in food quality control as well as the monitoring of the bee environment [56].

Honey is the result of a bio accumulative process that is useful to collect information about the environment within the bees' forage area. Bees are estimated to forage on plants growing in a relatively large area of more than 7 km^2 [8, 20].

If it is assumed that any hive includes at least 1000 worker bees and that each of them forages on 1000 flowers per day, the honey produced daily can be considered the outcome of at least one million interactions. In this way, the forage area is effectively sampled for trace elements and the concentration in honey of heavy and transition metals reflects their levels in the foraged area [8]. Presently, many countries are considering the use of honeybees to monitor environmental pollution. Those substances that potentially

could be monitored in the environment by honeybees include heavy metals, pesticides, veterinary drugs and radioactive substances.

However, the analysis of honey for trace elements content in the monitoring of the bee environment may be a difficult task because of low contaminant concentrations and complex interpretation of results [56].

1.12. Digestion of honey

Metal determination in sugar-rich foodstuffs has been a challenging analytical task because of the interference caused by the organic matrix [38].

Honey is a complex matrix consisting mostly carbohydrates, water and minor components (organic acids, enzymes, amino acids, vitamins, pigments). The direct FAAS analysis of an aqueous solution of honey sample is restricted because such a matrix, following pyrolysis in argon atmosphere, led to the progressive accumulation of a carbonaceous residue into the graphite atomizer, deteriorating both sensitivity and precision. For this reason, a wet digestion stage is generally recommended. This involves destruction of the organic matter by acids [56].

1.13. Scope and benefits of the study

The research concerning the determination of mineral content of honey is increasing during the last years [20].

Since Honey offer a potential dietary supplement and shows therapeutic features, it is important to know the levels of trace elements that are essential to health.

In this research work it was aimed to find some relationships among individual groups of honey and the correlation among individual constituents.

To the best of our knowledge, no study of trace heavy metals in Ethiopian honey has been made. Methodology and analysis data are available in the scientific literatures which refer to honey from other countries with different agro-ecology and botanical sources. Trace heavy metals are important from an economic stand point as they are not to exceed maximum limits regulated by importing countries such as the EU countries.

1.14. Objectives of the Study

General Objectives:

The overall objective of this project is to determine trace heavy metals and physicochemical parameters in the extracted and processed Ethiopian honey.

Specific Objectives:

1. To develop suitable wet digestion method for the extraction of metal ions from honey for their sub-sequent determination using flame atomic absorption spectrometry.

2. To determine the trace heavy metals: Cd, Pb, Zn, Co, Cu, Ni, Mn, Fe and Cr in honey using flame atomic absorption spectrometer for honey samples collected in Addis Ababa supermarkets and Beza-Agro Honey Processor (Adama).

3. To determine some physicochemical parameters such as pH, electrical conductivity, ash content of the honey samples collected in Addis Ababa supermarkets and Beza-Agro Honey Processor (Adama) and compare with national and international standards.

4. To evaluate the quality of some samples of honey from the market from the point of view of physicochemical properties and content of trace metals and comparing these samples with others, analyzed honeys in literature

2. Experimental

2.1 Equipment and reagents

2.1.1. Equipments

A refrigerator (Hitachi, Tokyo, Japan) was used to keep the collected samples and digested samples until analysis. A digital analytical balance (Mettler Toledo, Model AG204, Switzerland) with ± 0.0001 g precision was used to weigh honey samples. 2 mL, 5 mL, and 10 mL pipettes and a micropipette (DRAGONMED, Shanghai, China) were used for measuring different amounts of acids and standard solutions. 50 and 100 mL volumetric flasks were used to dilute sample solutions and prepare standard solutions. 250 mL round bottom flasks fitted with reflux condenser were used in Kjeldahl digestion block (Gallenhamp, England) apparatus to digest honey samples, spiked honey samples and blank solutions. BUCK SCIENTIFIC MODEL 210 VGP (East Norwalk, USA) atomic absorption spectrophotometer equipped with deuterium ark background correctors using air- acetylene flame was used for analysis of the digested honey samples for the metals Mn, Ni, Pb, Fe, Zn, Cu, Co, Cr and Cd.

2.1.2. Reagents and chemicals

Reagents that were used in the analysis were all analytical grade. 69-72 % HNO_3 and 70 % $HClO_4$ (FINE MUMBAI-391780, India) were used for digestion of honey samples. 30 % H_2O_2 was also used during optimization of honey samples. Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Mn, Ni, Pb, Fe, Zn, Cu, Co, Cr, Cd (BUCK SCIENTIFIC PURO-GRAPHIC^{tm}) were used for preparation of calibration standards and in the spiking experiments. Deionized water (chemically pure of 1.5 μscm^{-1} and below) was used throughout the experiment for sample preparation, dilution and rinsing apparatus prior to analysis.

2.2. Procedures

2.2.1. Apparatus cleaning

The cleaning of any apparatus to be used is of great importance because this research project is focusing on analysis of trace metals in trace level. Hence, the apparatus such as pipettes, volumetric flasks, measuring cylinder and digestion flasks were washed with detergents and tap water, rinsed with deionized water, soaked in diluted nitric acid for two days, rinsed with deionised water, dried at room temperature and kept in dust free place until needed for use.

2.2.2. Sample collection

Four different types of honey samples were collected from different supermarkets in Addis Ababa city and from the processor Beza agro industry in Adama city randomly in February- March, 2009. The honey samples were stored in glass jars. The honey samples were then kept in the refrigerator, until analysis. All the natural samples examined were processed honeys of random (mixed) floral type.

2.2.3. Sample preparation

In accordance with AOAC 920.180, honey samples to be examined were heated to 65 ^{0}C in a water bath until liquefied to permit easier handling and to decrease viscosity for more uniform distribution. The samples were then cooled and weighed for subsequent analysis [57].

2.2.4. Physicochemical property determinations

To determine the physicochemical parameters, the samples of honey were analyzed according to AOAC and Ethiopian honey specification methods [34, 57] in order to

determine, pH, electrical conductivity and ash content. Three replicates were used for each honey sample.

2.2.4.1. Determination of ash content

Samples were prepared according to 920,181 method of the A.O.A.C. Honey sample of 5 g was weighed accurately into a pre-weighed porcelain crucible and gently heated on a hot plate until the sample was turned in to black and dry and hence there was no danger of loss by foaming and overflowing. The sample was then ignited at 600 ^{0}C in a furnace (overnight) to constant weight. Then the samples were cooled in a desiccator and weighed [3, 34, 57].

2.2.4.2. pH and Electrical conductivity

The pH and electrical conductivity were measured by means of a pH-meter (pH /Ion level 2, Germany) and conductivity meter (HANNA Instruments, Portugal), respectively. 70 mL of deionized water (pH 7.0) was added to 10 g of honey and mixed thoroughly. Then after instrument calibration, the pH and EC were measured directly.

2.2.5. Digestion of honey samples

Exactly 0.5 g of honey sample was accurately weighed on a digital analytical balance and transferred quantitatively in to a 250 mL round bottom digestion flask. 4 mL of freshly prepared 1:1 mixture of conc. HNO_3 and conc. $HClO_4$ was added to the sample. The sample was swirled gently to homogenize the mixture then it was fitted to a reflux condenser and digested continuously for three hours on a Kjeldahl digestion block by setting the temperature dial at 8 (240 ^{0}C). Each honey sample was digested in triplicate and hence a total of twelve digest were made for the four types of honey samples. Then it was cooled to room temperature for 10 min without removing the condenser from the flask and for 10 min after removing the condenser. To the cooled solution deionized water was added to dissolve the precipitate formed on cooling and to minimize

dissolution of filter paper by the digest residue while filtering with Whatman®, (110 mm, diam), filter paper. The round bottom flasks were rinsed subsequently with deionized water in to 50 ml volumetric flasks. And finally the volumetric flasks were made up to the mark with deionized water. The digestion gave a clear colorless solution. Digestion of a reagent blank was also performed for correcting the effect of the blank in parallel with the honey samples keeping all digestion parameters the same. For the analysis of the honey samples six reagent blank samples were prepared. All the digested samples were stored in refrigerator until analysis using FAAS.

2.2.6. Determination of the metals

10 mg/L intermediate standard solutions of metals of interest were prepared from the atomic absorption spectroscopy standard stock solutions that contained 1000 mg/L. These secondary standards were diluted with deionized water to obtain four working standards of each metal, i.e. Mn, Cd Co, Cr, Zn, Ni, Pb, Fe and Cu and were analyzed with FAAS (BUCK SCIENTIFIC MODEL 210GP) equipped with deuterium arc background corrector and standard air-acetylene flame system after the instrumental operating conditions were optimized for maximum signal intensity of the instrument. Triplicate determinations were carried out on each sample. Hallow cathode lamp for each metal (Mn, Cd Co, Cr, Zn, Ni, Pb, Fe, and Cu) operated at the manufacturer's recommended conditions were used at its corresponding primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. The same analytical procedure was employed for the determination of elements in the digested blank solutions. The operating conditions for FAAS employed for each analyte are given in table 2

Table 2. Instrumental operating conditions for determination of metals in honey samples by using FAAS

element	Wavelength (nm)	Detection limit (mg/L)	Slit width (nm)	Lamp current (mA)
Cu	324.7	0.020	0.7	1.5
Zn	213.9	0.005	0.7	2.0
Mn	279.5	0.001	0.7	3.0
Ni	341.5	0.040	0.2	3.0
Fe	248.3	0.030	0.2	7.0
Co	240.7	0.050	0.2	4.5
Cr	357.9	0.050	0.7	2.0
Cd	228.9	0.005	0.7	2.0
Pb	283.2	0.100	0.7	2.0

2.2.7. Digestion of honey samples spiked with standard metals solution

To check the efficiency of the developed optimized digestion procedure, known amount of each metal was added from the
1000 mg/L of stock solution in to flasks containing 0.5 g honey sample. The procedure was as the following: in the first spiking the standards of four of the metals (7 µL of Cr, 16 µL of Ni, 85 µL of Fe and 13 µL of Cd) which were prepared from the intermediate 10 mg/L of the elements prior by taking 1 mL from the stock standard solutions containing 1000 mg /L of the metals were spiked at once in to triplicate round bottomed flasks containing 0.5 g of honey sample and similarly, the remaining metals (84 µL of Zn, 20 µL of Cu, 17 µL of Co and 4 µL of Mn) were spiked at once in to other triplicate round bottomed flasks containing 0.5 g of honey sample. Then, the spiked samples were digested simultaneously with the unspiked samples based on the optimized (developed)

digestion method for honey. Each sample was then determined for their respective spiked metals by atomic absorption spectrophotometer.

2.2.8. Method Detection Limit

Method detection limit (MDL) is defined as the minimum concentration of analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero, but it may not necessarily be quantified as an exact value [58]. The Method detection limit was calculated by multiplying the standard deviation of the blank concentration by three. The results are shown in table 7.

3. Results and Discussion

3.1. Calibration of the Instrument

Atomic absorption spectrophotometer (BUCK SCIENTIFIC MODEL 210VGP**)** was used to determine trace metals concentration. Calibration of the instrument was done by the standards prepared before the determinations were done. The standards were prepared from the intermediate 10 mg/L of the metals which were prepared prior by taking 1 mL from the stock standard solutions containing 1000 mg /L, in 2% HNO_3, of the metals. After making sure that the instrument was properly calibrated, concentration of metals in each sample was measured. The correlation coefficient (R) of the calibration curves of each element was determined by plotting prepared standards concentration versus their corresponding absorbance. The standards and their corresponding correlation coefficients are given in table 3. The linear correlation coefficients obtained ranged between 0.99942 - 0.99999. The calibration graphs of each of the metals analyzed is shown in Figure 2.

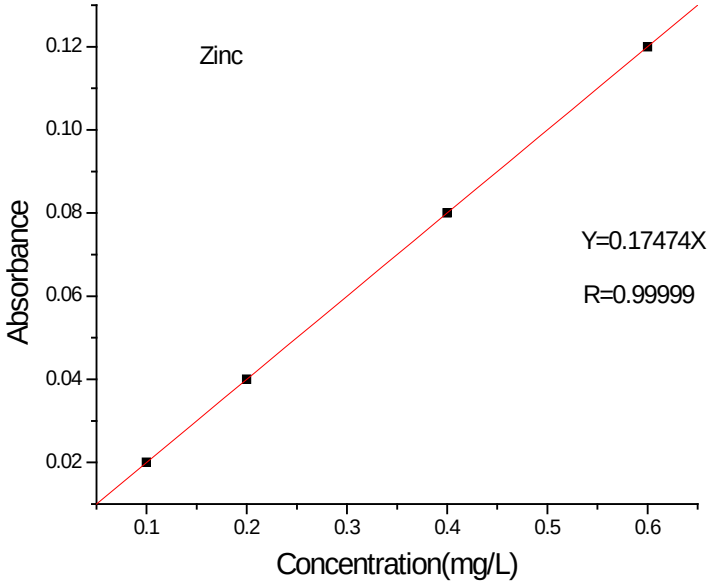

Figure 2a. Calibration curve of Zn

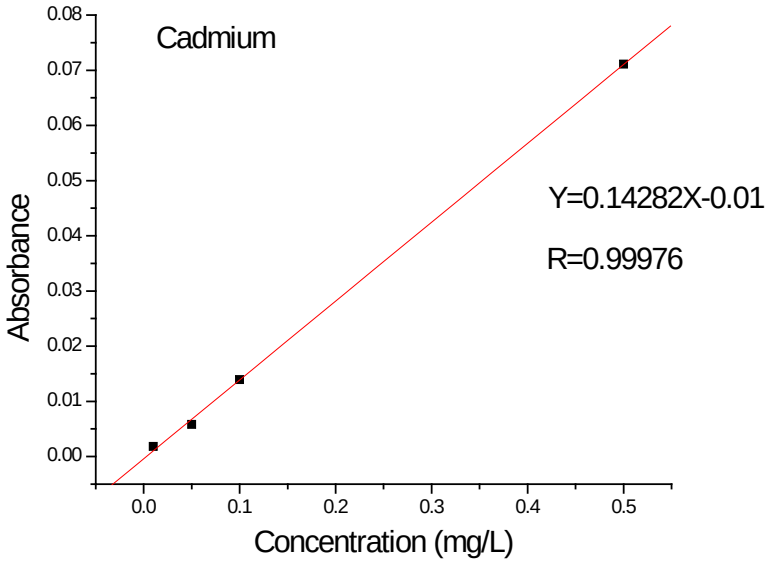

Figure 2b. Calibration curve of Cd

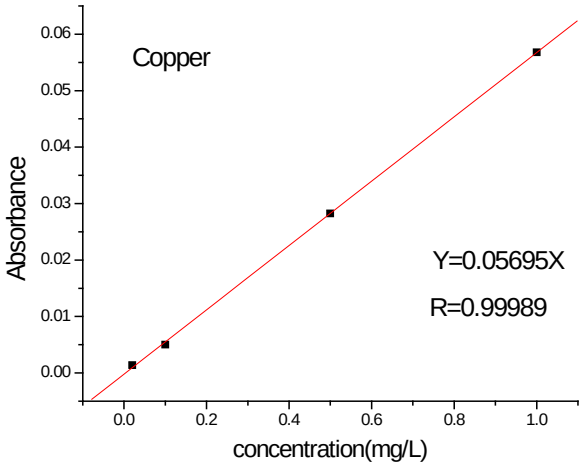

Figure 2c. Calibration curve of Cu

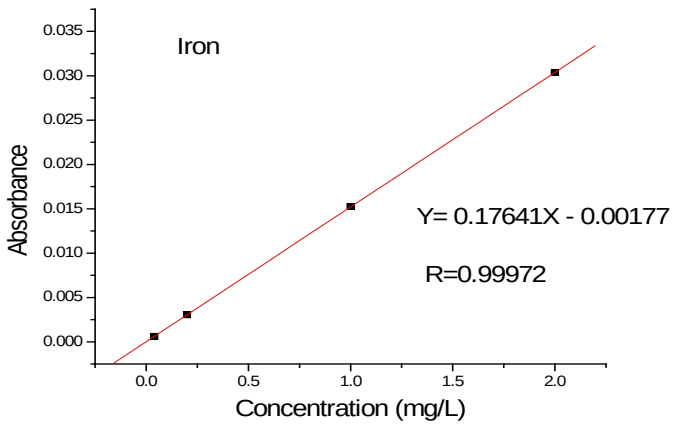

Figure 2d. Calibration curve Fe

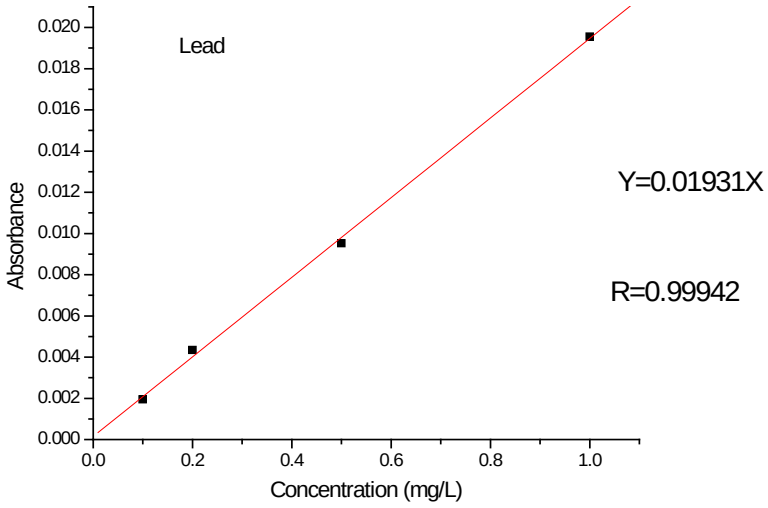

Figure 2e. Calibration curve of Pb

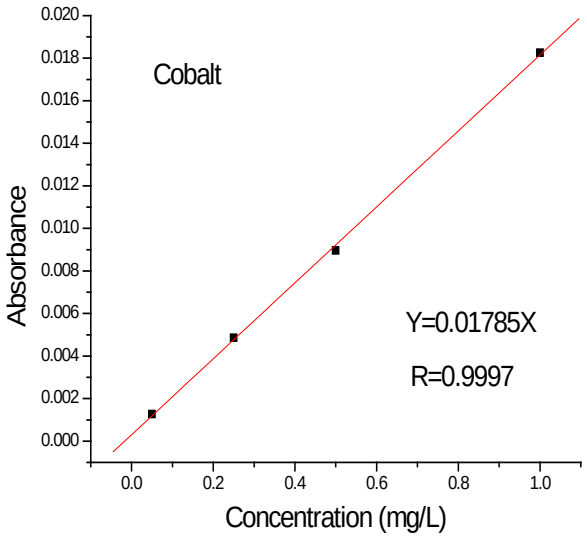

Figure 2f. Calibration curve of Co

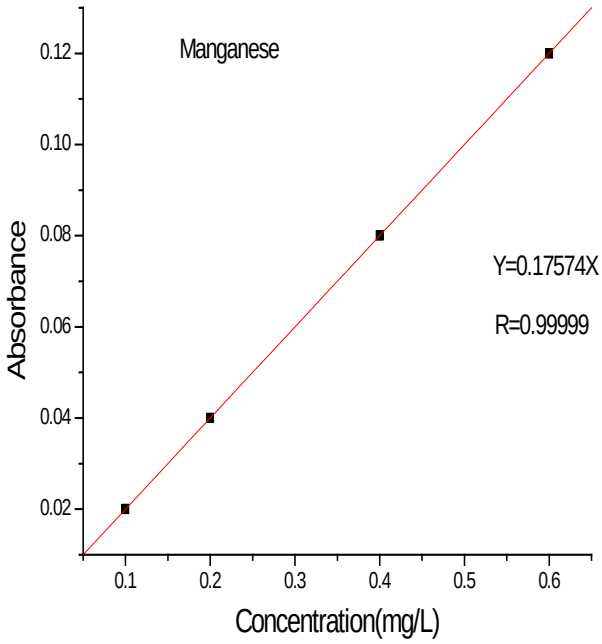

Figure 2g. Calibration curve of Mn

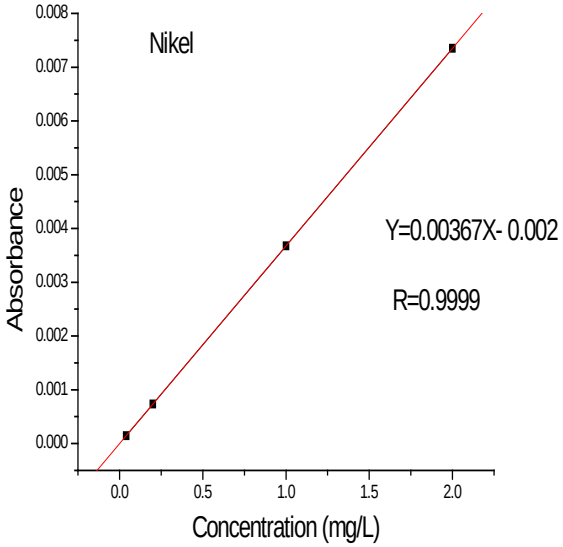

Figure 2h. Calibration curve of Ni

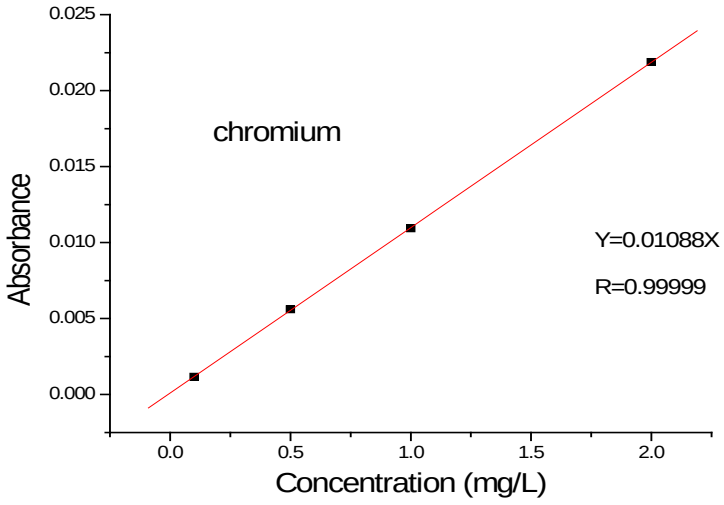

Figure 2i. Calibration curve of Cr

Table 3. Concentrations of the standard solutions used to establish calibration graphs for the determination of metals in honey samples and their corresponding correlation coefficients.

No	Metal	Concentration of intermediate standard (mg/L)	Concentration of standards (mg/L)	Correlation coefficient of calibration curves
1	Cu	10	0.02, 0.1, 0.5, 1	0.99989
2	Zn	10	0.02, 0.1, 0.2, 1	0.99999
3	Mn	10	0.1, 0.2, 0.4, 0.6	0.99999
4	Ni	10	0.05, 0.25, 1.25, 2.5	0.99999
5	Fe	10	0.5, 1, 1.5, 3	0.99972
6	Co	10	0.05, 0.25, 0.5, 1	0.99970
7	Cr	10	0.1, 0.5, 1, 2	0.99972
8	Cd	1 0	0.025, 0.05, 0.125, 0.5	0.99998
9	Pb	10	0.1, 0.2, 0.5, 1	0.99942

3.2. Physicochemical Parameters of Honey

3.2.1. Ash content

Honey normally has low ash content and it depends on the materials collected by the bees foraging on the flora [20, 59]. This variable was calculated adapting 920, 181 method A.O.A.C and the ES [34, 57].

Ash (% by mass) = W_1- W_2/ M

Where,

W_1 = weight of empty crucible

W_2 = weight of the ash and crucible

M = mass of the sample taken for the test

Table 4. Ash content of the of honey samples

Honey type	[a]Ash content (%w/w)
Tutu	0.31 ± 0.0133
Yeshi	0.17 ± 0.0018
Beza	0.46 ± 0.004
Dima	0.20 ± 0.003

[a]values are mean ± SD of triplicate honey samples

The ash contents in the specifications of Ethiopian, European and Codex Alimnetarius standards are ------- respectively. Thefore the ash contents in all analyzed honey brands were below the limit (0.6 %) allowed by different standards [6, 8, 27].

The highest value of ash content was recorded for Beza honey samples (0.46 %), followed by Tutu (0.31 %), Dima (0.20 %), and Yeshi (0.17 %). Beza honey exhibited almost twice as much element as light honey samples Dima and Yeshi. This result was obtained as expected. It is known that the mineral content is closely related to the electrical conductivity, i.e. the higher the mineral content is the higher the electrical conductivity. The ash content and the electrical conductivity were also related to the honey type, i.e. floral or honeydew, being higher in the latter [59].

In this study it was found that samples that had high ash percentages manifested the highest electrical conductivity. The relationship between ash percentage and electrical conductivity are important because they permit an indirect estimate of mineral content [18].

3.2.2. pH and EC

pH and electrical conductivity of solution of 10 g of honey in 75 mL of deionized water was measured using the instruments pH meter and conductivity meter, respectively. The results are displayed in table 5 below.

Table 5. pH and Electrical conductivity of the four honey brands

Honey type	pH	[b]EC (ms/cm)
Tutu	4.109± 0.0335	0.12 ± 0.020817
Yeshi	4.314 ±0.0066	0.1 ± 0.01
Beza	4.333 ± 0.115	0.29 ± 0.01
Dima	4.329 ± 0.009	0.11 ± 0.01

[b]EC electrical conductivity values calculated as mean ± SD of triplicate honey samples

Pure honey is characterized by a conductance near zero. In the Codex Alimentarius, the maximum electrical conductivity for pure floral honey is 0.8 $mscm^{-1}$ [6].

It was reported that if honey is adulterated with water or saturated sugar solutions, it will display greater conductance than pure honey [3].

The electrical conductance of the four honey samples were found considerably lower than that established by the Codex Alimentarius (< 0.8 $mscm^{-1}$) [6].

As can be seen from table 5 the highest electrical conductivity was recorded for Beza (0.29 ± 0.01) honey followed by Tutu (0.12 ± 0.021), Dima (0.11 ± 0.01) and Yeshi (0.1 ± 0.01). This is in the same order with that of the ash content as expected because the higher ash content is the higher electrical conductivity.

The pH values of all honey brands were found to be acidic and the values were also comparable with each other, i.e. there was only slight pH difference among the samples analyzed. The mean pH values recorded were: 4.10, 4.31, 4.32, and 4.33 for Tutu, Yeshi, Dima and Beza honey, respectively. The pH was also within the accepted range (3.5 – 5.5) according to the same standard [6]. The pH values of each honey type are given in table 5.

From Tables 4 and 5, the ranges of physicochemical properties analyzed could be summarized as: pH 4.11 - 4.45, ash content 0.17 - 0.46 % and electrical conductivity of 0.10 - 0.29 $mscm^{-1}$ which were all with in the limits established by different national and international standards [6, 27, 34].

3.3. Optimization of Digestion Procedure

Extraction is still one of the most critical steps during honey analysis. Basically, the extraction should be performed in such a way that the analyte is separated from the interfering matrix without loss, contamination, or change of speciation and with minimum interference [7].

Series of digestion procedures were involved to optimize digestion of honey at different conditions by varying digestion time, reagent volume, volume ratio of reagents and digestion temperature. Accordingly, fifteen digestion procedures were tested for the digestion of honey sample. The optimization of digestion procedure is given in table 6. The optimum procedure was selected depending on: minimum reagent volume consumption, digestion time, obtaining clear solution, complexity and simplicity. Finally the optimal procedure was chosen on the basis of these criteria requiring three hours for complete digestion of 0.5 g honey sample with 2.0 mL of 65 - 70% HNO_3 and 2.0 mL 70% $HClO_4$ as given in Table 6.

Table 6. Optimization of Digestion Procedure

No	Amount of honey (g)	Volume of Reagents	Digestion temperature (^{0}C)	Digestion time (h)	Result after filtration and dilution
1	0.5	6 mL HNO_3	120	3	Deep Yellow clear solution
2	0.5	5 mL HNO_3	120	3	Yellow clear solution
3	0.5	4 mL HNO_3	120	3	Yellow clear solution
4	0.5	4 mL HNO_3	120	3	Yellow clear solution
5	0.5	4 mL HNO_3	180	3	Yellow clear solution
6	0.5	4 mL HNO_3	240	3	Pale Yellow clear solution
7	0.5	2:2 mL (H_2O_2:HNO_3)	240	2 h	Yellow clear solution
8	0.5	2:2 mL (H_2O_2:HNO_3)	240	2.5	Pale clear solution
9	0.5	2:2 mL (H_2O_2:HNO_3)	240	3.5	Colorless clear solution
10	0.5	2:2 mL ($HClO_4$:HNO_3)	240	2	Pale Yellow clear solution
11	0.5	2:2 mL ($HClO_4$:HNO_3)	240	2.5	Pale Yellow clear solution
12	*0.5*	*2:2 mL ($HClO_4$:HNO_3)*	*240*	*3.0*	*Colorless clear solution (Optimum)*
13	0.5	2:2 mL ($HClO_4$:HNO_3)	180	2.5	Yellow clear solution
14	0.5	2:2 mL ($HClO_4$:HNO_3)	240	2.5	Pale Yellow clear solution
15	0.5	2:2 mL ($HClO_4$:HNO_3)	300	2.5	Colorless clear solution

The common drawbacks of other tested procedures were their higher chemical composition, longer duration for complete digestion and observation of colors in some of the tested procedures.

Due to the use of acid digestion procedure, the acid used for the digestion might add some amount of metals to the samples to be analyzed since most of the reagents have metals as impurities. So, it is always necessary to prepare acid/reagent blanks for each

type of digestion performed. Thus, reagent blanks were prepared with the same reagents and subjected to the same digestion procedure as the samples.

3.4. Precision and Accuracy

The precision of an analytical procedure expresses the closeness or agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of an analytical procedure is usually expressed as the variance, standard deviation or percent relative standard deviation (relative coefficient of variation) of a set of measurements.

For this research project the precision of the results were evaluated by the standard deviation and percent relative standard deviation of the results of four samples (n = 4) and triplicate readings for each sample, i.e. a total of 9 measurements for a given bulk sample. The results of the present analysis are reported with the corresponding standard deviation and percent relative standard deviation of nine measurements for a bulk sample. Table 9 shows the result and % RSD of each metal in each honey types.

3.5. Method detection limit

Usually MDL is defined as the amount of analyte that gives a signal equal to three times the standard deviation of the blank [60]. In other words, method detection limit is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically three times the noise. For the present study, Six reagent blank solution were digested and each of the samples were analyzed for metal concentrations of Mn, Fe, Cu, Zn, Mn, Co, Ni, Pb and Cd by AAS. The standard deviations for each element were calculated from the six blank measurements to determine method detection limit. The detection limits were obtained by multiplying the standard deviation of the reagent blank by three.

Table 7**.** Method detection limit of elements (3δblank, n = 6) determined using the blank solution.

Element	Zn	Cu	Cox	Cd	Cr	Fe	Mn	Ni	Pb
[a]MDL (µg/g)	0.081	0.07	0.08	0.06	0.05	0.09	0.03	0.09	0.05

[a] Method detection limit

The data in table 7 clearly show that the method developed is applicable to determine the metal concentration in the honey samples at trace levels (µg/g).

3.6. Recovery test

Method validation is the process of providing that analytical method is acceptable for its intended purpose. The validity of the analytical procedure can be checked by: (a) analyzing a series of samples using two different methods, e.g. the new method and a standard method; (b) analyzing reference or certified reference material; (c) performing standard methods of analysis; (d) comparative analysis with other experienced laboratories; and (e) analyzing spiked samples.

For this project study, standard method of analysis [6, 27, 34] were used to determine some physicochemical properties such as: ash content, electrical conductivity and pH of honey and the results found were compared with the scientific literatures which refer to honey from other countries with different agro-ecology and floral (botanical) sources.

Since there is no certified reference material for honey in our laboratory, the validity of the optimized digestion procedure for honey was also checked by carrying out spiking. As can be seen from table 8 the percentage recovery for honey samples are between 93 to 104%, which are within the acceptable range for all metals. The percentage recovery was calculated by using the following formula given below [61].

$$\%\,\mathrm{Recovery} = \frac{C(spiked) - C(non-spiked)}{C(added)} \times 100\%$$

Where: C(spiked) is metal content of the spiked sample

C(non-spiked) metal content of non spiked sample

C(added) is metal content of metal added

Table 8. Recovery Values of metals for the analyzed honey samples

Metal	[a]Conc. in sample (µg/g)	Amount added (µg/g)	[b]Conc. in spiked sample (µg/g)	% [c]Recovery
Zn	4.22 ± 1.02	1.69	5.89 ± 0.55	98.9 ± 1.5
Co	0.83 ± 0.02	0.332	1.12 ± 1.6	93 ± 5.5
Cd	0.67 ± 0.06	0.268	0.93 ± 0.8	97 ± 8.1
Cr	3.32 ± 0 .09	1.328987	4.60 ± 1.3	95.7 ± 2.2
Fe	8.53 ± 4.12	3.412	12.1 ±1.0	104.1 ± 1.3
Ni	0.8 ± 0.05	0.32	1.1 ± 0.01	100.5 ± 5.4
Pb	[d]ND	-	-	-
Mn	0.18 ± 0.011	0.072	0.25 ± 0.6	97.2 ± 4.1
Cu	0.091 ± 0.08	0.036	0.125 ± 0.7	93.4 ± 2.2

[a] value are mean ± SD of triplicate readings of triplicate samples

[b] Values are mean ± SD of triplicate readings of triplicate samples

[c] mean recovery ± SD of percentage recoveries of triplicate reading of triplicate samples

[d] not detected , concentration is below detection limit

3.7. Determination of the concentration of metals in different honey brands.

The concentrations of nine trace metals in some commercially available Ethiopian honey brands were determined by using FAAS. The result showed that the metal content of each honey brand is different from one to the other. Of the nine elements determined seven were found above the detection limit in all of the four honey brands. Cd was not detected in one honey type (Tutu honey) as it was found below the detection limit, i.e. < 0.06 µg/g where as Pb was not detected in all of the honey types (< 0.05 µg/g). Therefore the commercially available honeys are free from the non essential toxic metal Pb where as the other none essential toxic metal Cd was found at very trace level in the three honey types and none in one honey type. The concentrations of the metals in the four honey brands are summarized in table 9 with their corresponding % RSD.

Table 9 concentration (µg/g) of trace metals in four honey type samples.

Metal	Average concentration of metals ± SD and their corresponding % RSD							
	Beza	% RSD	Dima	% [a]RSD	Tutu	% RSD	Yeshi	% RSD
Zn	1.92 ± 0.18	9.4	4.22 ±0.31	7.3	4.17 ± 0.41	9.8	1.10 ± 0.09	8.1
Co	1.17 ± 0.08	6.8	0.83 ± 0.02	2.4	0.98 ± 0.08	8.1	0.60 ± 0.05	8.3
Cd	0.19 ± 0.01	5.2	0.67 ± 0.06	8.9	[b]ND	-	0.54 ± 0.05	9.2
Fe	12.43 ± 1.0	8.1	8.53 ± 0.7	8.2	12.3 ± 0.97	7.8	5.37 ± 0.36	6.7
Ni	4.46 ± 0. 30	6.7	0.8 ± 0.01	1.25	1.24 ± 0.1	8.1	3.96 ± 0.25	6.3
Cr	4.33 ± 0.33	7.6	3.32 ± 0.22	6.6	3.828 ±0.36	9.4	1.20 ± 0.10	8.3

Pb	[b]ND	-	[b]ND	-	[b]ND	-	[b]ND	-
Mn	0.88 ± 0.07	7.9	0.18 ± 0.011	6.1	0.16 ± 0.01	6.2	0.64 ± 0.06	9.3
Cu	0.46 ± 0.04	7.6	0.091 ± 0.08	8.7	0.362 ± 0.02	5.5	0.09 ± 0.006	6.6

[a] percent relative standard deviation

[b]not detected

.

3.8. Distribution of metals in honey samples

Essential trace metals are widely distributed in honey samples. Each honey brand analyzed was found to have different metal contents. Generally, Beza Honey was found to be richest in its trace mineral content followed by Dima and Tutu Honey. Where as Yeshi Honey was found with the least mineral content. The results of this study also indicated that iron has the highest concentration followed by nickel, chromium, zinc, cobalt, manganese, cadmium and copper where as lead was found bellow detection limit.

The concentration of the metals in each honey brand is described clearly below.

3.8.1. Concentration of metals in Beza honey

Beza honey contains Fe in the highest amount with concentration of 12.43 ± 1.01 µg/g followed by Ni (4.46 ± 0.31 µg/g), Cr (4.33 ± 0.37 µg/g), Zn (1.92 ± 0.18 µg/g), Co (1.17 ± 0.08 µg/g), Cu (0.885 ± 0.07 µg/g) and Mn (0.467 ± 0.36 µg/g). Where as Cd (0.19 ± 0.51 µg/g) was found to have the least concentration as shown in the figure 6 below. Lead was observed to be below the detection limit, i.e. < 0.05 µg/g. These results showed that the essential trace metals are found at higher amount where as the non essential in a lower amount or none.

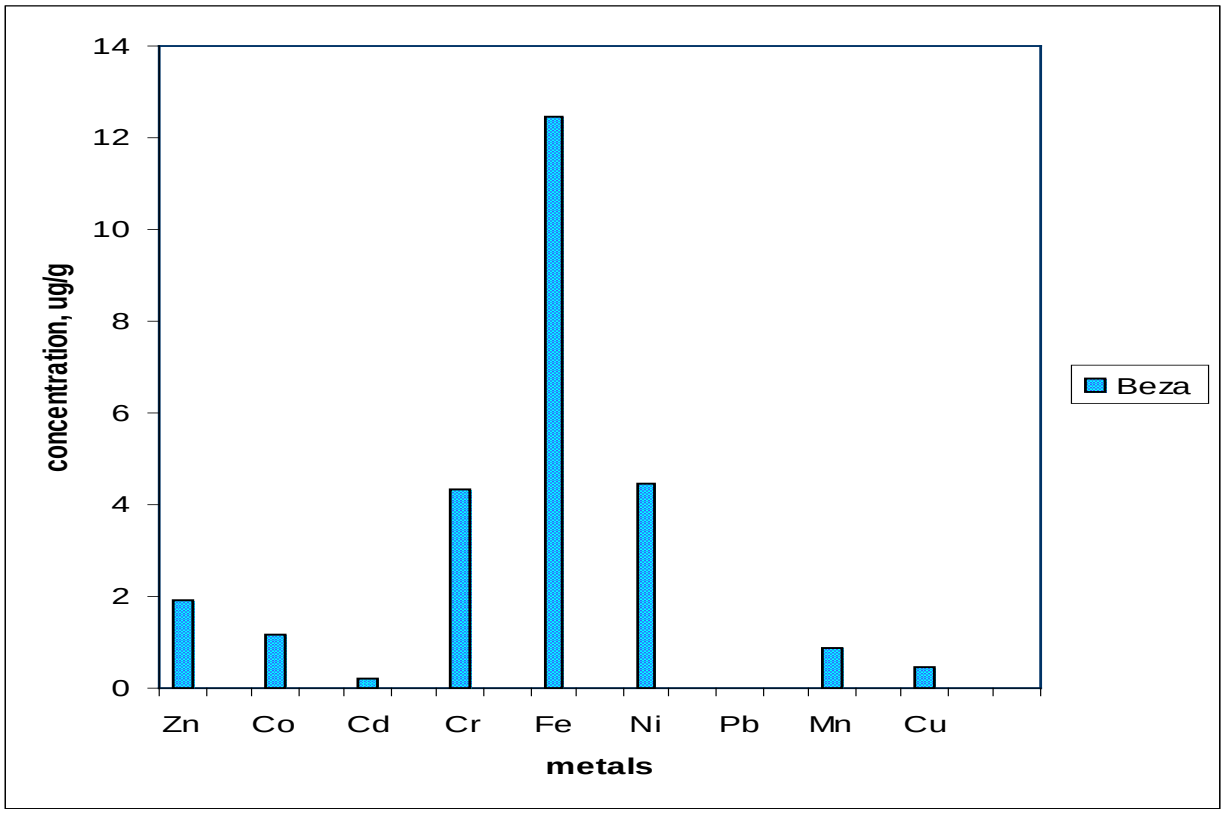

Figure 3. Shows concentrations of metals in Beza honey

3.8.2. Concentration of metals in Dima honey

Dima honey was another honey type analysed and as it can be seen in the figure x below, generally, the concentration of the metals in this brand was found to have the same trend as the previous brand. It included the following average concentrations (µg/g) in the decreasing order: Fe (8.53 ± 0.7), Zn (4.22 ± 0.31), Cr (3.32 ± 0 .09), Co (0.83 ± 0.02), Ni (0.8 ± 0.01), Cd (0.67 ± 0.06), Mn (0.18), Cu (0.091), but similarly, Pb was not detected as it was found below the detection limit.

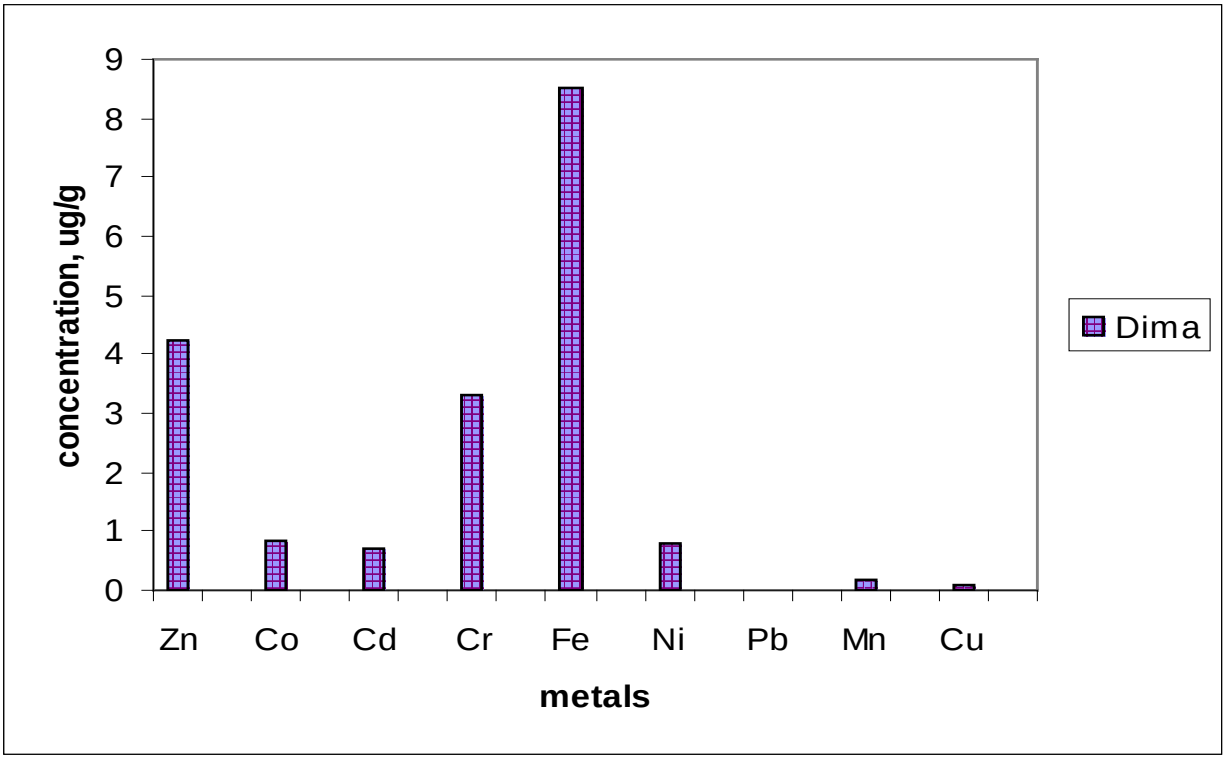

Figure 4. Shows Concentrations of metals in Dima honey

3.8.3. Concentration of metals in Tutu Honey

The third type of honey sample whose trace metals analyzed was Tutu honey. The general trend of the content of the metals was not much different from the previous two brands. The concentrations are displayed in figure 8 below.

This honey is also very rich in its content of Fe (12.3 ± 0.97± 4.6 µg/g), followed by Zn (4.17 ± 0.41), Cr (3.82 ± 0.36 µg/g), Ni (1.24 ± 0.01 µg/g), Co (0.98 ± 0.08 µg/g), Cu (0.362 ± 0.02 µg/g) and Mn with the least concentration (0.16 ± 0.01 µg/g). In contrast to the three honey brands, not only Pb but also Cd was found below detection limit, i.e. < 0.06 µg/g.

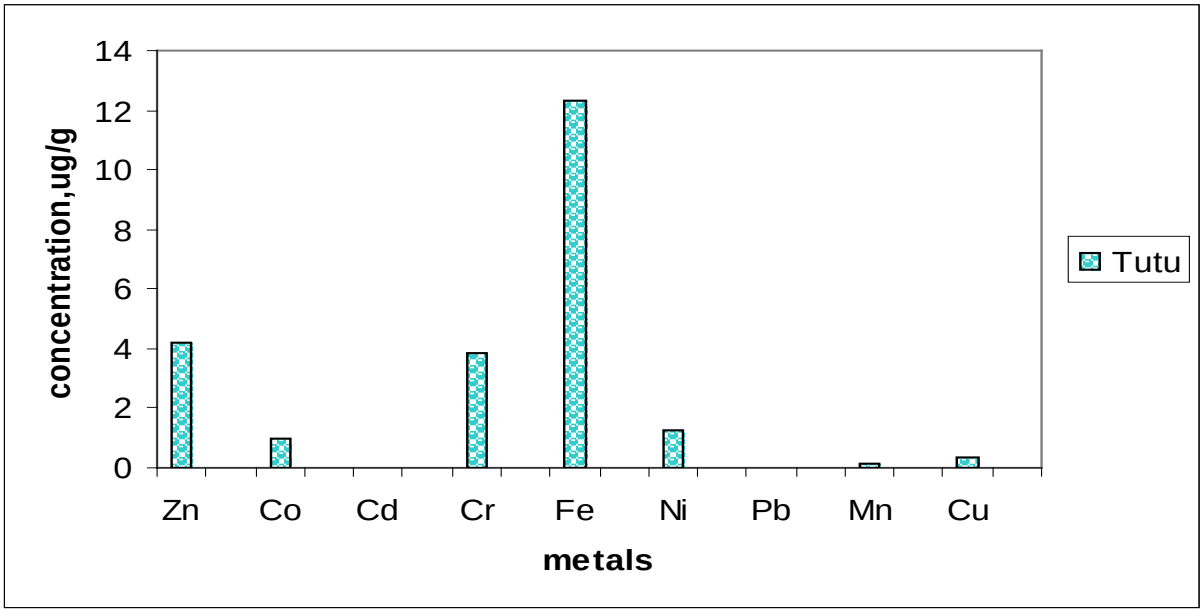

Figure 5. Shows Concentrations of metals in Tutu honey

3.8.4. Concentration of metals in Yeshi honey

The fourth honey sample studied was Yeshi. Compared to other honey brands, Yeshi honey was found with the least amount of the corresponding metals except for the metals Ni, Mn and Cd. But generally, the level of metals followed the same trend as the previous three honey brands.

The concentrations of the metals for this honey were found in the following decreasing order: Fe (5.37 ± 0.36 µg/g), Ni (3.96 ± 0.25µg/g), Zn (1.1 ± 0.09 µg/g), Cr (1.20 ± 0.10 µg/g), Co (1.17 ± 0.08 µg/g), and Mn (0.64 ± 0.06), Cd (0.19 ± 0.51 µg/g) and Cu (0.09 ±0.006). Like wise, the non essential trace heavy metal lead was not detected in Yeshi honey samples. The concentration of the metals is shown in figure 9 below.

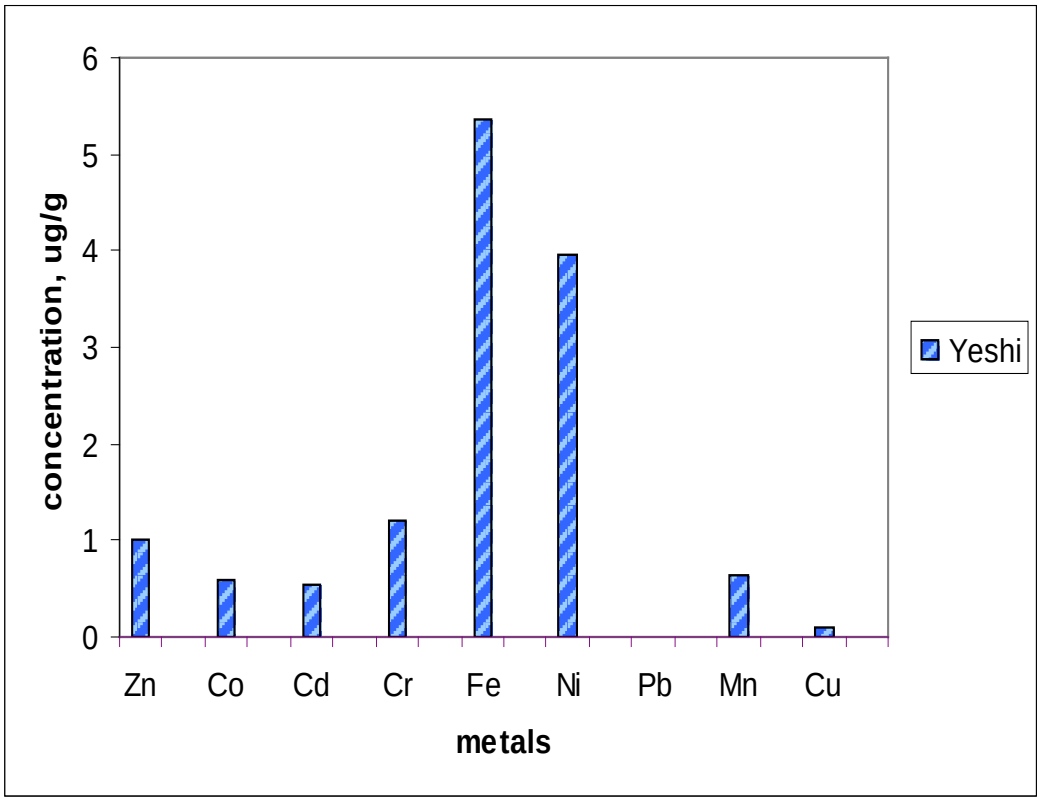

Figure 6. Shows Concentrations of metals in Yeshi Honey

3.8.5. Comparison of the concentration of metals in four different honey brands

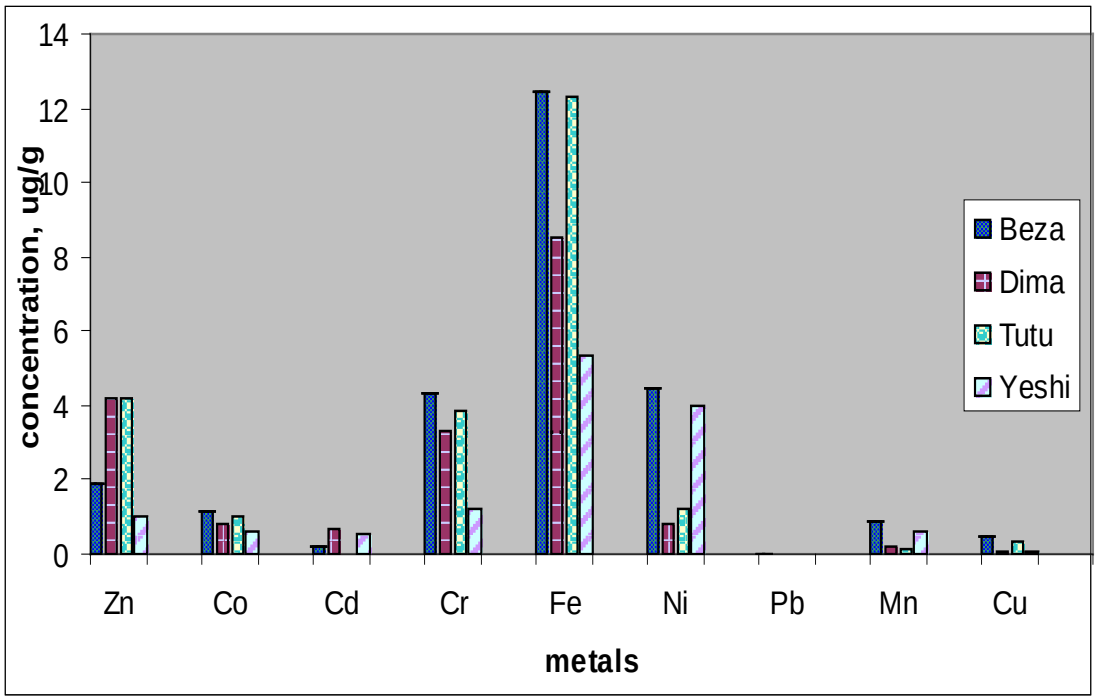

Figure 7. Concentration of metals in Beza, Dima, Tutu and Yeshi honey

Even though the trend of levels of metals concentration in each brand is similar, the metal content of each brand is found to be quite different. Table 10 and the bar graphs of figure 10 below the rank of each honey brand from the first to the fourth for their metal contents.

Table 10. Order for concentration level of metals in the honey samples

Metal	Order for concentration level			
	1st	2nd	3rd	4th
Fe	Beza	Tutu	Dima	Yeshi
Ni	Beza	Yeshi	Tutu	Dima
Cr	Beza	Tutu	Dima	Yeshi
Zn	Dima	Tutu	Beza	Yeshi

Co	Beza	Tutu	Dima	Yeshi
Mn	Beza	Yeshi	Dima	Tutu
Cd	Dima	Beza	Yeshi	Tutu
Cu	Beza	Tutu	Dima	Tutu

These show that there is considerable variation in mineral composition among honey samples of different origins.

The data variation is probably due to the floral type, the botanical origin, storage conditions anthropogenic factor, season of the year, rain fall and so on.

From the figures and the tables discussed so far one can summarize that in general Beza honey has the highest amount of essential trace metals followed by Tutu , Dima and finally by Yeshi. Unlike to the three honey brands (Beza , Dima and Yeshi), the non essential toxic metal Cd was too low to be detected in one honey brand (Tutu Honey) where as in the rest honey brands, it was found in very minute amount below the maximum values allowed according to FAO and WHO [62]. The other non essential toxic heavy metal Pb was found too low to be detected in all honey brands.

All the trace metals analyzed in the four Ethiopian honey brands were found below maximum tolerable limits [8, 62, 63]. And hence are in safety baseline levels for human consumption.

As can be seen from figure 10 Fe was found in highest amount with mean concentration ranging from 5.37 to 12.43 µg/g followed by Ni with mean concentration range of 0.8 to 4.46 µg/g , Cr (1.20 – 4.33 µg/g) , Zn (1.92 - 4.22 µg/g), Co (0.60 -1.17 µg/g), Cd (ND – 0.69 µg/g), Mn (0.16- 0.885 µg/g) and Cu (0.09- 0.4676 µg/g). The non essential metal Pb was not detected.

3.9. Comparison of metal content and physicochemical properties of honey with other reported values

Although various chemical analysis target to a similar objective, there may be a difference in sampling, sample preparation and analysis techniques. Considering all these, the result of the present study can be compared to the findings of other authors.

Different reports indicated that the metal content, EC, pH and ash content of honey of different botanical origin [3, 7, 8, 10, 11, 15, 18, 20, 29, 30, 46, 64, 65].The comparisons made for metal content, pH, electrical conductivity and ash content are shown in table12, 13, 14 and 15, respectively.

Table 11. Comparison of the concentration of metals in honey of the present study with other reported values [3, 7, 8, 11, 18, 20, 30, 46, 64].

	Concentration of Metals (µg/g)				
Country	Fe	Ni	Zn	Cr	Co
Chile[18]	0.1-6.36	0.01-1.04	0.01-4.73	0.03 - 1.92	0.03-0.60
Pakistan[3]	4.35 - 7.54	1.02 - 1.48	1.98 - 2.94		0.84 - 1.12
Brazil [7]	1.50 -6.24		ND-0.94		
Switzerland[11]	0.136 - 9.85	0.001- 1.966	0.016- 4.133	0.001- 0.037	
Turkey[30]	1.1-5.2		1.1-24.2		
Venezuela[64]	3.5-39				
Morocco[20]	0.88-207.6		0.04-2.74		
Ethiopia (present study)	5.37-12.43	0.8 - 4.46	1.92 - 4.22	1.20 -4.33	0.60 - 1.17

Country	Concentration of Metals (µg/g)			
	Cd	Cu	Mn	Pb
Saudi Arabia[8]	0.038- 0.080	0.206-0.389	0.188-0.373	0.002-0.037
Chile[18]	0.01 -0.05	0.06 -2..00	0.01-3.14	0.01-0.11
Brazil [7]	NDR	ND	ND -3.0	NDR
Switzerland[11]	0.001- 0.026	0.051- 3.317	0.125- 12.354	0.003- 0.329
Turkey[30]	0.010 -0.02	0.25-1.10	0.18-1.21	0.017- 0.030
Venezuela [64]		0.3-1.67	0.4-1.67	
Morocco[20]	0.0013-0.0249	0.51-4.75	0.080-9.76	0.036-1.88
Ethiopia (present study)	ND-0.69	ND – 0.4676	ND-0.885	ND

[a]not detected

[b]not determined

In the present study Fe, Ni and Cd were at slightly higher concentration than those found in Chilean and Turkish honeys. However Fe was within the concentration range found in Moroccan and Venezuelan honeys. The levels of Cu, Zn and Mn were in a very good agreement, i.e. within the values found in the countries: Chile, Turkey, Venezuela, Brazil, Switzerland honeys where as Co and Ni are comparable with the reports from Pakistan honey.

The non essential metal Pb in this study was found below the detection limit, i.e. < 0.05 µg/g and this is in a very good agreement with most of the results reported from different countries [1, 3, 6, 7, 8, 10]. Except honey from Tutu, however, the other non essential metal Cd was found slightly higher than most of the reported results. This might need further studies on the geographical origin to help in finding out possible sources of heavy metal pollution and vegetation of the area from where the honey was originated.

In general, the results obtained in this study were remarkably in a good agreement with those reported from other parts of the world implying acceptability and validity of this work regardless of some factors contributing deviation in some ways.

Table 12. Reports of pH of honey from different countries [8, 10, 15, 18, 29, 65]

Country	pH
Saudi Arabia [8]	3.88 – 4.25
Czech Republic [10]	3.70 – 4.40
Algeria [63]	3.40 – 6.23
Chile [18]	3.79 - 5.08
Croatia [29]	4.21- 5.55
Argentina [15]	3.19 - 4.06
Ethiopia (present study	4.11 - 4.45

Table 13. Reports of electrical conductivity of honey from different countries [7, 8, 10, 11, 15, 18, 65]

Country	[a]EC
Saudi Arabia 1[8]	0.139 – 0.398
Czech Republic [10]	0.239 - 0.613
Algeria[65]	0.21 – 1.24
Chile [18]	0.11- 0.97
Brazil[7]	0.25 – 1.07
Switzerland[11]	0.10- 0.50
Croatia [29]	0.45 -0.89
Argentina[15]	0.113 - 0.278

Ethiopia (present study	0.1 - 0.29

[a]electrical conductivity

Table 14. Reports of ash content of honey from different countries [7, 8, 10, 64]

Country	Ash content(%w/w)
Czech Republic [10]	0.14 - 0.58
Saudi Arabia [8]	0.11 – 3.398
Brazil[7]	0.04-0.76
Venezuela[64]	0.352- 0.643
Ethiopia (present study	0.17-0.46

As can be seen in table 13, 14 and 15, the present studied physicochemical properties: pH, EC and ash content are also remarkably in a good agreement with the reported results from different countries in the world.

4.0. Statistical analysis

Analysis of variance (ANOVA) is a powerful statistical method which is used to identify the source of variation of more than one means obtained for different experiments. Since the sample means vary from one sample to another analysis of variance is used to test whether there is significance difference or not between samples mean thus enabling the source of errors. For the present study, the significance of variation within sample and between samples has been studied using ANOVA. It can also be made on a computer using excel and SPSS (SPSS13.0 for windows, The Apache Software foundation, 2000)

software was used for the statistical analysis. For the present study SPSS software was used to calculate the presence or absence of significant difference in mean concentration of each metal between four brands of Ethiopian honey namely, Beza, Dima, Tutu and Yeshi Honey. The following results were obtained.

For Co, no significance difference at 95% confidence interval ($P \geq 0.05$) was observed in the mean concentrations between all the four honey samples. The mean concentrations of Zn do not differ significantly ($P \geq 0.05$) for Dima and Tutu while it differs significantly for Dima and Tutu compared to Yeshi and Beza. Fe mean concentrations do not also vary significantly ($P \geq 0.05$) for Beza and Tutu while it differs significantly for Beza and Tutu compared to Dima and Yeshi. Similarly, the mean concentrations of Cu do not vary significantly for Dima and Yeshi but it differ significantly ($P \geq 0.05$) for Dima and Yeshi compared to Beza and Tutu. The others differ significantly for ($P \geq 0.05$).

The mean concentrations of Zn do not vary significantly ($P < 0.05$) for Beza and Yeshi and also for Dima and Tutu. In the case of Cd there is no significant difference between three honey brands Dima, Yeshi and Beza.

In a similar manner, in the case of Cr significant difference ($P < 0.05$) occurs between each honey brand except between Dima and Tutu, and Beza and Tutu.

For Zn, Ni and Mn there was no significant ($P < 0.05$) difference in means between Beza and Yeshi and between Dima and Tutu where as there is significant difference ($P < 0.05$) when Beza and Yeshi are compared to Dima and Tutu.

The analysis of variance showed that there is significant variation in levels of elements between each brand of honey. The difference may be due to the floral type, the botanical origin, storage conditions anthropogenic factor, season of the year, rain fall and so on.

4. Conclusions and Recommendations

1. In this work, study such as physicochemical properties: ash content , Electrical conductivity and pH values by using furnace, conductivity meter and pH- meter , respectively and contents of nine trace metals using FAAS has been determined on the commercially available Ethiopian different honey brands.

2. The optimized wet digestion method for honey analysis was found efficient for the metals analyzed and it was validated through the recovery experiment and a good percentage recovery was obtained (93-104%).

3. Statistical analysis (ANOVA) results suggested that there were significant variation in the level of some elements between the honey brands, which could be attributed to different factors such as geographical origin of the honey, different precaution taken during processing, storage conditions and floral source. For some elements the variations were insignificant, which could be attributed to having similar factors mentioned above.

4. The metals content and the physicochemical properties investigated in honey samples were found within the ranges established by national and international standards.

5. The levels of metals in honey samples, determined and assessed for its quality comparing with permissible limits given by various agencies and organizations, showed that the present studied trace metals. Fe, Cu, Co, Zn, Mn, Ni, Pb and Cd levels are in safety baseline levels for human consumption. The study also showed that the analyzed metals were found to follow the decreasing order; Fe > Ni > Cr > Zn > Co > Cd > Mn > Cu

6. The non essential toxic metal Pb in the present study was found below the detection limit, i.e. < 0.05 µg/g and this is in a very good agreement with most of

the results reported from different countries. Although the amount of Cd detected was in a safe amount for human consumption, it was found slightly higher than most of the reported results. This might need further studies on the geographical origin to help in finding out possible sources of heavy metal pollution and vegetation of the area from where the honey was originated.

7. In general, the results obtained in this study are remarkably in a good agreement with those reported from other parts of the world implying acceptability and validity of this work regardless of some factors contributing deviation in some ways.

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