Addis Ababa University

Research and Graduate Programs

Department of Chemistry

Determination of major and trace metals in date palm fruit (*Phoenix dactilifera*) samples using flame atomic absorption spectrophotometry (FAAS)

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July 2020

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERISITY

RESEARCH AND GRADUATE PROGRAMS

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A thesis submitted to the Department of chemistry Addis Ababa university in partial fulfillment of the requirements for the degree of master of science in chemistry.

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Approval of the Thesis

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RESEARCH AND GRADUATE PROGRAMS

I declare that this project entitled "Determination of major and trace metals in date palm fruit (*Phoenix dactilifera L.,*) samples using flame atomic absorption spectrophotometry (FAAS)" is my original work under the supervision of Dr. Merid Tessema at the Chemistry Department of Addis Ababa University. This paper has not been submitted or presented in any other university, college or institution.

Name: Feven Tamirat

Signature: ______________________

Date: ______________________

July 2020

DEDICATION

DEDICATED TO MY FAMILY

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List of abbreviation

ANOVA	Analysis of variance
FAAS	Flame atomic absorption spectrophotometry
FAO	Food and Agriculture Organization
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-OES	Inductively coupled plasma-optical emission spectrometry
MDL	Method detection limit
MQL	Method quantification limit
NIST	National Institute of standards and technology
MP-AES	Microwave plasma-atomic emission spectroscopy
RDA/AI	Recommended dietary allowance/ Adequate intake
RSD	Relative standard deviation
SD	Standard deviation

Abstract

The aim of this study was to investigate the levels of major and trace metals in date palm fruit samples from Afar, Iraq, and Saudi Arabia. Levels of selected metals (Na, Ca, Fe, Zn, Ni, Mn, Cu, Cd and Pb were determined by flame atomic absorption spectrophotometry (FAAS). Known weight of oven dried date palm fruit samples were wet digested using 3 mL HNO_3 and 1 mL $HClO_4$ for 3 hours at a temperature 300 ^{o}C.

The mean concentration range (mg/kg) of each metal in date palm fruit sample were Ca (205.5-299), Na (134-320), Fe (38.8-115), Ni (25.1-42.2), Zn (9.27-27.9), Mn (7.11-16.3), Cu (0.002-1.15). Cd and Pb were not detected.

The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with standard solutions prepared and the percentage recoveries varied from 91.6 % to 97.8 % which is an acceptable range.

The mean concentration of metals in the three types of date palm fruit samples indicated higher concentration of Ca in date palm fruit from Afar and Saudi Arabia. Higher concentration of Na was found in date palm fruit from Iraq as compared to the Afar and Saudi Arabia date palm fruit samples. ANOVA indicated that there is no significant difference among the sample means.

Person correlation coefficients of metals from date palm fruit between Fe-Na, Na-Zn, Na-Mn, Ca-Zn, Fe-Ni, Fe-Mn, Mn-Ni showed very strong correlation while Ni-Na, Fe-Cu, Cu-Ni showed strong negative. Na-Cu, Ca-Fe, Ca-Ni, Ca- Mn, Ca-Cu, Fe-Zn, Ni-Zn, Zn-Mn, Zn-Cu showed moderate, weak, and very weak correlation.

Keywords: Date palm fruit, Digestion, Optimization, Major metals, Trace metals

1. Introduction

1.1 Background of the study

The date palm (*Phoenix dactylifera*) tree belongs to the family *Arecaceae* and is considered as a symbol of life in the desert, as it tolerates high temperatures, water stress, and salinity more than many other fruit crops (Lunde, 1978 and Wekesa *et al.*, 2008). Date fruit has tremendous nutritional value due to its rich content of essential nutrients including carbohydrates, salts, minerals, dietary fibers, vitamins, fatty acids, amino acids, and proteins. The date fruit is unique and characterized by certain distinct properties (Barreveld, 1993). One of the major features that makes it special is that it is the only fruit eaten as a dietary staple in the world and has remained for thousands of years as a daily diet for millions of people. Another unique property of date palm fruit is at each of the three major maturity stages such as *khalal* (fresh, hard ripe), *rutab* (crisp to the succulent), or *tamr* (fully ripe stage), dates can be devoured. These terms are the base on changes of color and nutrient compositions of date fruit. To reach the fully ripened or *tamr* stage, it takes 150–200 days after pollination.

At *tamr* stage, date fruits have the lowest amount of moisture and are ready for shelf preserving. Fruits are fully ripe, very sweet, dark brown or nearly black, soft and chewy, with the lowest moisture and rich in reducing sugars fructose, glucose, and sucrose (Sawaya *et al*., 1983; Aleid *et al*., 1999). The *tamr* stage is the final stage of the date maturation. At this stage, microorganisms will not be able to grow, however, moisture uptake and compositional changes can occur when there is no preservation during storage. Dates are classified according to their moisture content. The 'soft date' contains more than 30% moisture. The 'semi-dry' dates have moisture contents ranging between 20 – 30%, and 'dry dates' have less than 20% moisture (El-badawy, 2001).

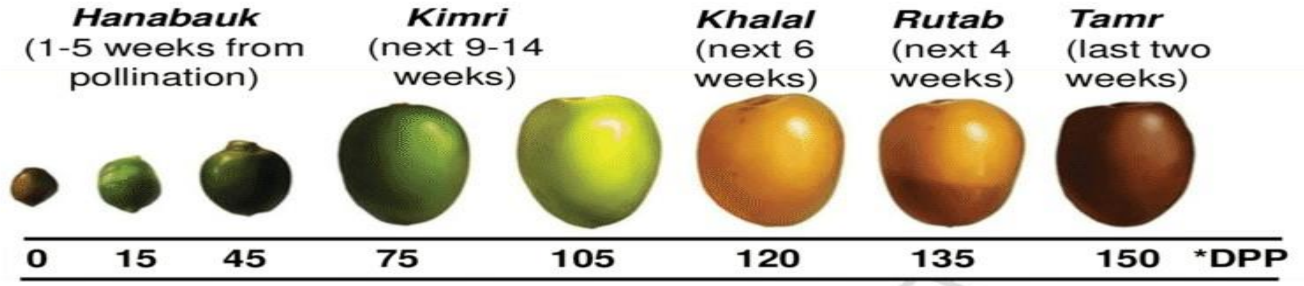

Figure 1. Different fruiting stages of date palm according to *DPP (days post pollination) showing *Khalal*, *Rutab*, and *Tamr* the three edible stages of the fruit (Al-Mssallem *et al*., 2013).

Dates have always played an important role in the diet of people from many countries, and the consumption of dates is particularly popular in the Middle East, North Africa and South Asian countries, where about 90% of the global production of dates takes place (Benmeddour *et al*., 2013). The Food and Agriculture Organization of the United Nations (FAO) has been actively engaged in developing the cultivation of the date palm. It recognizes social, economic and ecological importance in countries, with suitable agro-climatic conditions, where it is traditionally grown (Hodder *et al*., 2016). Ethiopia currently imports date fruits from different date growing countries by paying hard currency.

The date palm has been introduced to Ethiopia from Middle East countries about 200 years ago by traders from Yemen and Sudan (Demeli *et al*., 2013). From that moment on it is cultivated mainly by agricultural producers in Afar, Somali, Gambella, Dira Dawa, and Benishangul-Gumuz Regions. The production of date palm especially in Afar Region has a long tradition where it is established as wild crop in Afambo, Aysaita, Gewane, and Amibara districts along the Awash River (Salah, 2015) within the Danakil Depression which are especially suitable for date palm production (Hussen, 2010).

Furthermore, the presence of Awash River in the region is an excellent opportunity for establishment and expansion of small-scale and large plantation of date palm in the region (Salah, 2015). Historically, the Afar people have trade connections with the Arab world as a result of their geographic location. Islamic history indicates that the first immigration of the Prophet's followers to 'Ardul Habasha' went through the Afar regions indicating the historic link that they had with the Arab world dating as far back as 1400 years ago.Date plantation in the region was inherited from the Arabs.

The Afar have established date farms in areas around Awsa in Afambo District. These farms produce date fruits mainly for local consumption. Ethiopia has high demand for date fruit mainly during the month of Ramadan creating high demand for date fruit during this month and other religious ceremonies and traditional rituals (Hussen, 2010). There are more than 5000 date palm species popular all over the globe (Bashah, 1996).

They differ by size, color, texture, antioxidant activity, phenolic content and, so on. Therefore, depending on the nutritional and mechanical characteristics of the ancient superfruit it is possible to use it for various purposes. The main goal of this study is to determine the levels of major and trace metals of date palm fruit (*Phoenix dactylifera*) from three different areas namely Afar, Iraq and Saudi Arabia and to assess the sugar content in these various types.

1.2 Statement of the problem

Date palm production is the most important agricultural activity for hot arid regions of the world that improves foreign exchange earnings of countries in such harsh environments (Botes *et al*., 2002). The date palm also plays an important role in the ecology of various desert and semi-desert environments by providing a shelter to the ground thereby helping to protect the soil against harsh weather, and improve the soil fertility by conserving water. Most of the Ethiopia date palm are from the Afar regional state because the region has a suitable landscape for date farming.

Moreover, dates are a good energy source largely because of their high sugar content. While dates have the lowest protein and fat content compared to other dried fruits, they can still provide high carbohydrates and energy for our diet.

Thus, dates make a significant contribution to the diet and hence their contribution to nutrition is important. Several kinds of research have been conducted in many countries about the ancient fruit in the determination of the elemental contents (Sueleman *et al*., 2014) and determination of heavy metals (Aldjain *et al*., 2011) of the date palm fruit by using different kinds of spectroscopic techniques. Therefore, the main aim to conduct this research is to determine the essential, non-essential and trace metals of the three specific types of the *Phoenix dactilifera* by using flame atomic absorption spectrophotometry (FAAS) and also to determine the sugar contents that is found in the *Phoenix dactilifera by* using refractometry. So far, no research has been conducted using the flame atomic absorption spectrophotometry (FAAS) for elemental determination and refractometric technique for sugar content determination in Ethiopia.

1.3 Objective of the study

1.3.1 General objective

This research aims to determine the levels of Na, Ca, Ni, Zn, Fe, Cu, Mn, Pb, and Cd metals and the determination of sugar contents in the different types of selected date palm fruits (*Phoenix dactilifera)* which are currently available in Addis Ababa market.

1.3.2 Specific objectives

- To determine the levels of Na, Ca, Ni, Zn, Fe, Cu, Mn, Pb, and Cd metals in date Palm fruit in the different samples collected using flame atomic absorption spectrophotometry.
- To compare the contents of metals selected in the three different types of date palm fruit among each other.
- To determine the level of sugar content for each type of date palm fruit.

2. Literature review

2.1 The general description of date palm fruit

2.1.1 The history of date palm fruit

The date palm, *Phoenix dactylifera* is one of fifteen species in the genus *Phoenix* of the palm family (*Arecaceae*, previously *Palmae*) distributed in the subtropics and tropics (Henderson, 2009). Even though its exact place of origin is unknown due to long cultivation, it probably originated from the Fertile Crescent region between Egypt and Mesopotamia. The species is widely cultivated throughout North Africa, the Middle East, the Horn of Africa, and South Asia, and is naturalized throughout the world in many tropical and subtropical areas.

Figure 2. Date palm trees grown at Awash river bank in Alassabolo kebele of Afambo district (photo taken during observation of the production areas, 2016) (Lemlem *et al*., 2018).

The date palm tree that has been in cultivation since 2400 BC was praised and cherished as is evident from the drawings and sculptures of ancient civilizations of the Sumerians, Assyrians, Babylonians, and Egyptians, and later by the Greeks and the Romans that inhabited the Mediterranean basin where date palm and other *Phoenix* species are also commonly grown (Pruessner, 1920). Date palm still carries great religious significance in all three major religions of the world. In Islam, date palm is cited 21 times in the Holy Quran and 300 times in the Hadith of the Prophet Mohammed, making it by far the most frequently cited plant. Similarly, date palm is praised in Christian and Judaism faiths and has been linked to numerous religious ceremonies (Musselman, 2007).

2.1.2 The botanical description of the date palm fruit

The date palm is a tall evergreen tree that has unbranched palms which can grow up to 30 m (Jatoi *et al*., 2009). The date palm trunk, also called stem or stipe is vertical, cylindrical, and columnar of the same width. Depending on the variety, age of a palm and environmental conditions, leaves of a date palm are 3 to 6 m long (4 m average) and have a normal life of 3 to 7 years (Zaid *et al*., 1999). It is a dioecious plant group that includes distinct male and female plants. Only the female trees produce fruit, but one male tree can produce enough pollen to pollinate 40-50 female trees. Unripe dates are green in color, maturing to yellow, then reddish-brown when fully ripe. A single large bunch may contain more than a thousand dates and can weigh between 6 to 8 kg. Each tree produces between five and ten bunches. A mature female tree can produce more than 150 pounds of fruit annually. Date palms begin to bear fruit at 3 to 5 years and are fully mature at 12 years (Jatoi *et al*., 2009).

2.1.3 Different types of date palm fruit

Three are about 5000 varieties of the date palm fruit that grow around the world. These different types have been developed for thousands of years. Among these various types below are some of the popular date varieties in market demand.

Medjool Dates - are grown in Saudi Arabia, Morocco, Palestine and Jordan. They are soft and delicious sweet dates with a rich test and fibrous texture which embraces a reddish-brown color. Also due to their high moisture they are perfect for eating fresh.

Barhi Dates - were introduced to California in 1913 from Basra, Iraq. These dates are fresh and also rich with a syrupy flavor. They have a firm skin and flesh turn amber at rutab stage then the color turns to golden brown when stored.

Piarom Dates - are cultivated throughout the Persian Gulf and the Middle East since ancient Mesopotamia 6,000 BC. They are suitable for people with diabetes and blood pressure. They are semi dry, long, thin and oval in shape with a dark brown to black skin and they have a unique sweet taste.

Thoory Dates - Thoory is famous in Algeria. These dates are called the bread date due to their perfect pairing with bakeries. The fruit is medium to large oblong in shape with golden-brown flesh and is fairly dry.

Halawy Dates - The Halawy date variety is from Mesopotamia, and the name literally means “Sweet” in Arabic. Halawy is a small to medium fruit with golden-brown skin and flesh that is delicate and tender. It has a sweet caramel honey like flavor with a soft texture.

2.1.4 Worldwide production of the date palm fruit

The production of date palms worldwide is an increasing phenomenon. Date palm production is the most important agricultural activity for hot arid regions of the world that improves foreign exchange earnings of countries in such harsh environment (Botes *et al*., 2002). Egypt is the world leader in date production and cultivation among the leading date producing countries. This country produces approximately 1,084,529 metric tons of dates per year. Since 1993, Egypt has increased date cultivation by more than 100% and now has an estimated 15,582,000 date palm trees. Following Egypt, Iran produces 947,809 metric tons annually. The majority of Iran’s date exports go to Asian countries.

The biggest importers are India (16%) and Malaysia (11%). Russia follows by importing 9.9% of Iran’s exported dates. Saudi Arabia is the third-largest producer of dates. This country has the perfect conditions for date growth and cultivation, producing 836,983 metric tons per year. This country is exporting about 8.8 percent of the world's dates that totals around $94.3 million. The main importers of these exports are Jordan (19%), Yemen (17%), and Kuwait (15%). Iraq produces 675.440 metric tons per year and accounts for 7.3 percent of global date exports. This country once yielded more than 1 million metric tons a year and had 30 million date palms. That was in the 1980’s, however, before the war with Iran. During the regime of Saddam Hussein, the annual output reached only 420,000 tons. The government has since reinvested in the industry, and production is slowly increasing. The country exported dates worth $77.5 million in 2014, 79% of which went to India (Amber, 2017).

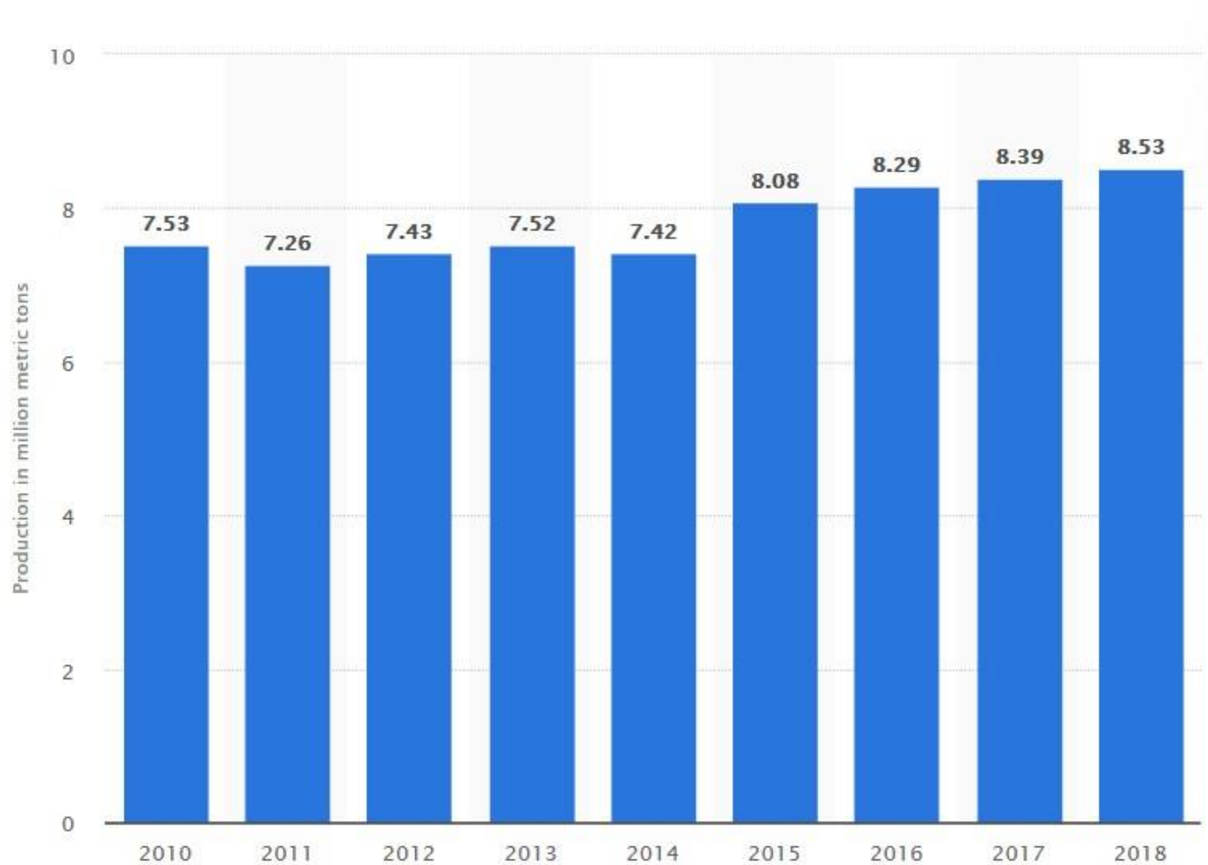

Figure 3. This statistic illustrates the production of dates worldwide from 2010 to 2018, in million metric tons. In 2018, the global production volume of dates amounted to about 8.53 million metric tons, up from 7.53 million metric tons in 2010 (Shahbandeh *et al*., 2020).

2.1.5 The occurrence of metals in the date palm fruit

2.1.5.1 Trace metals

Trace metals, are micronutrients that are required, in very low concentrations. Trace elements such as iron, iodine, fluoride, copper, zinc, chromium, selenium, manganese, and molybdenum are vital for maintaining health. Also referred to as microminerals, these trace elements are part of enzymes, hormones, and cells in the body (Sukhsatej *et al*., 2018). Trace elements function primarily as catalysts in enzyme systems; some metallic ions, such as iron and copper, participate in oxidation-reduction reactions in energy metabolism. Iron, as a constituent of hemoglobin and myoglobin, also plays a vital role in the transport of oxygen (National Research Council, 1989).

Manganese

Manganese is found as a free element in nature; it is often found in minerals in combination with iron (Mejia *et al.*, 2013). It is a transition metal with a multifaceted array of industrial alloy uses, particularly in stainless steel. Manganese not only helps in enzyme formation but is also necessary for activation. It functions as an antioxidant, helps to grow bones and heals wounds by increasing the development of collagen. Good manganese sources include pineapple, nuts, and beans.

Iron

Iron is present in all body cells (Sukhsatej *et al.*, 2018). It plays an important role in plants for chlorophyll growth and in animals it is a component of hemoglobin that carries oxygen from the lungs into the body's tissues. Iron is an important nutrient in our diet. Iron deficiency, the most common nutritional deficiency, can cause anemia and fatigue that affects the ability to perform physical work in adults (Vhanda, 2013).

Cobalt

Cobalt is a naturally occurring element that is found in most animals, plants, water, soil, and rocks typically in a trace amount. Usually, it is found in the environment combined with other elements such as oxygen, sulfur, and arsenic. Also, cyanocobalamin or vitamin B12 is biochemically an important compound that is essential for good health in animals and humans. Cobalt and its compounds are used in the manufacturing of ceramics and industrial production of paints. Cobalt deficiency results in the disruption of vitamin B12 synthesis which may cause anemia and the risk of developmental abnormalities. An excess intake of this metal has its own effects such as fibrosis in lungs and asthma.

Nickel

Nickel is a hard, malleable, and ductile metal which is a fairly good conductor of heat and electricity. It is mainly used in the preparation of alloys in which nickel alloys are known by strength, resistance to corrosion, and heat. Nickel is commonly applied as an ingredient of steel and other metal products. It can be present in common metal products such as jewelry.

A small quantity of nickel is essential to our body but when the uptake is too high it can result in health effects such as heart disorders, respiratory failure, lung cancer, and as such.

Copper

Copper is one of the few metals that can occur in nature in a directly usable metallic form (Mc Henry, 1992). It is often used in the production of switches, transformers, electrical conductors, and telecommunication due to its ductility, electrical and thermal conductivity. It is present in small quantities in the human body and helps the body perform the functions required in the brain, blood, and more. In plants also it is a constituent and activator of several enzymes such as ascorbic acid oxidase and lactase. Low levels of copper can affect the immune system and energy levels.

Zinc

Zinc is an essential metal, necessary for sustaining all life. It is a trace element in the diet, forming an essential part of many enzymes, and playing an important role in protein synthesis and cell division (Kumar *et al*., 2019). Zinc occurs naturally in air, water, and soil, but zinc concentrations are increasing unnaturally as a result of human activities. Mostly zinc is used during industrial activities, such as mining, waste combustion, and steel manufacturing. Zinc is needed for the proper growth and maintenance of the human body. It is found in several systems and biological reactions, and it is needed for immune function and it can be obtained from meat, seafood, dairy products, nuts, legumes, and whole grains that offer relatively high levels of zinc. Zinc deficiency in humans is caused by reduced dietary intake, inadequate absorption, increased loss, or increased body system utilization.

2.1.5.2 Essential metals

Essential metals which are also known as macronutrients are nutrients that are required in greater quantities by plants and other living organisms. These usually contain water, carbohydrates, fat, and protein. They are also called nutrients which provide energy. Elements that are categorized under macronutrients are carbon, hydrogen, nitrogen, oxygen, phosphorus, potassium, calcium, sulfur, and magnesium. Energy is essential for the body to grow, repair, and develop new tissues, conduct nerve impulses, and regulate the life process.

Our body requires food that is rich in nutrients for proper body growth and work. In the absence of healthy food, numerous health conditions, and deficiency diseases collide in our bodies. Some of the common outcomes due to nutritional deficiencies are marasmus, kwashiorkor, goiter, anemia, night blindness, and as such. Some of the essential metals are discussed below.

Sodium

Sodium is an essential mineral that occurs naturally in foods such as milk, beets, carrots, meat, and spinach. The largest source of sodium in the diet comes from sodium chloride or table salt. sodium helps to maintain the balance of the physical fluids system. It is also required for muscle and nerve functioning. Too much intake of sodium can damage our kidney and increases the chances of high blood pressure. The deficiency of sodium also leads to muscle cramps, fatigue, or low energy, weakness, and Hyponatremia (low blood sodium).

Magnesium

Magnesium is an essential metal that is crucial to the body's function. Magnesium helps maintain the blood pressure to be normal, the heart to have a steady rhythm, the bone to be strong and it is also required for the proper function of muscles, nerves, and other parts of the body. Low levels of magnesium in the body have been linked to diseases such as high blood pressure, heart disease, diabetes, and stroke. Magnesium is known for a long time as the lighter structural metal in the industry, due to its low weight and to its capability of forming mechanically resistant alloys (Korla *et al*., 2020).

Aluminum

Aluminum is a natural content that is found in fruits and vegetables. The most commonly used foods that contain substantial amounts of aluminum containing food additives are baking powders, pancake mixes, cake mixes, and pickled vegetables (Lione, 1983). It is carried throughout the bloodstream to the kidney, where it is quickly discharged but for patients with a known history of kidney failure, the high intake may accumulate and result in a toxic effect. Aluminum compounds are used to make vaccines more efficient and aluminum hydroxide is used to treat stomach ulcers and aluminum salts are also generally used in cosmetics for deodorants.

Potassium

Potassium is required by all living organisms. It helps to maintain fluid balance, muscle contractions, and nerve signals. The body needs a delicate balance of potassium to help the heart to function properly and other muscles as well. But if the potassium level is too much it can lead to dangerous, and possibly deadly, changes in heart rhythm and also with a risk of high blood pressure, heart disease, stroke, arthritis, cancer, digestive disorders. The greatest demand for potassium compounds is in fertilizers.

Calcium

Calcium is an essential metal that is necessary for life. Calcium allows our blood to clot, our muscles to contract, and our heart to beat, in addition to building bones and maintaining them healthy. About 99% of the calcium in our bodies is in our bones and teeth. Dairy products, such as milk, yogurt, and cheese are high in calcium. The deficiency of calcium causes disruptions of the metabolic rate and other bodily dysfunctions such as chest pains, numbness in fingers and toes, muscle cramps, brittle nails, dry skin, and tooth decay (Cashmere, 2018).

Foods with high levels of oxalic acid such as sweet potatoes, beans, spinach bind to calcium and inhibit its absorption. So, to maximize the absorption of calcium in the body using calcium supplements can be the other option other than using dairy products and others. calcium supplements help add calcium to the diet.

Chromium

Chromium is an essential nutrient that is required for fat and sugar metabolism. It forms a compound in the body that seems to develop the effects of lower glucose levels and insulin. Chromium deficiency causes impaired glucose tolerance. As well as, large doses of chromium as a supplement can cause lower blood sugar, kidney and liver damage, and stomach problems. Chromium is used in stainless steel and other alloys, Chromium compounds are valued as pigment for yellow, red, and orange colors.

2.1.5.3 Non-essential metals

Metals have very diverse uses and play a significant role in the industry dominated human society. This rapid urbanization has triggered heavy metals to contaminate the environment. Heavy metals are commonly known for their environmental pollution due to their toxicity persistence in the environment, and bio accumulative nature. Their natural sources include weathering of metal-bearing rocks and volcanic eruptions while mining and various industrial and agricultural activities are anthropogenic sources. Heavy metals such as cadmium, gallium, germanium, indium, lead, mercury is considered non-essential metals.

Lead

Lead is a naturally occurring element found in small amounts in the earth's crust. While it has some beneficial uses, it can be toxic to humans and animals, causing health effects (Debnath *et al*., 2019). Due to human activities such as industrial facilities, usage of lead-based paint, the manufacturing of ceramics, batteries, gasoline, and cosmetics there is much exposure to lead in the environment. Lead also has important properties like softness, malleability, ductility, poor conductibility, and resistance to corrosion. Exposure to high levels of lead may cause anemia, weakness, and kidney and brain damage. Very high lead exposure can cause death (Fries, 2015).

Cadmium

Cadmium and its compounds are highly toxic and exposure to this metal is known to cause cancer and targets the body's cardiovascular, renal, gastrointestinal, neurological, reproductive, and respiratory systems (Howard *et al*., 2001). Cadmium is used in rechargeable nickel-cadmium batteries and can be used in other products including mobile phones, cordless power tools, cameras, computers, and lights.

Also, nuclear reactor rods are often used to hold nuclear fission reactions under control due to their capacity to absorb neutrons. Cadmium waste from industries is concentrated primarily in the soil, with small amounts entering the air by combustion of fossil fuels and water by industrial waste.

Mercury

Mercury is a naturally occurring element that is found in air, water, and soil (Lam, 2017). It is the only metal that is liquid at room temperature. It is used in making thermometer, barometer, and a component for dental amalgam for making fillings for teeth also used in the chemical industry as a catalyst.

The high amount of mercury in the body can lead to permanent neurological changes and developmental problems in the brain as well as small amounts of mercury may cause serious health problems. Therefore, mercury may have toxic effects on the nervous, digestive, and immune systems, and lungs, kidneys, skin, and eyes (Lam, 2017).

2.2 The medicinal benefits of the date palm fruit

Date palm trees have been growing in harsh climatic conditions for the last 5000 years and playing a vital role as a source of energy, nutrition security, and as a healthy fruit. The nutritional value of dates is ideally a high energy food as they have a high content of sugar (Al-orf *et al*., 2012).

Among the various health benefits of dates, the insoluble and soluble fiber found in dates helps to clean out the gastrointestinal system, allowing the colon to function at higher efficiency rates. The mature dates contain potassium, which is known as an effective way to control diarrhea.

The vitamins present in dates makes it an ideal boost to the health and functionality of the nervous system. It makes date a superfood to strengthen the bones and fight against painful and debilitating bone diseases because it has a rich source of minerals such as selenium, manganese, copper, and magnesium, all of which are integral to healthy bone growth.

Dates have medicinal uses including anticancer, antihyperlipidemic, hepatoprotective activities and thereby serving as an essential healthy food in the human diet (Biglari *et al*., 2009).

The presence of pharmacological properties could be due to the presence of high concentrations of minerals and a variety of other phytochemicals of diverse chemical structure (Baliga *et al*., 2011).

Furthermore, dates contain an impressive level of iron which makes them the perfect home remedy for the treatment of iron deficiency. One of the most interesting facets of dates is the presence of organic sulfur in them.

2.3 The side effects of the date palm fruit

Dates have various types of health benefits as presented in the previous section. However, dates can also be harmful if the consumption level is very high. They can not be helpful to people with diabetes because dates have high calorific value and are rich source of natural sugars such as glucose, fructose, and sucrose. Dates are naturally fiber rich therefore if there is an excess fiber that is consumed it can give rise to constipation.

It is also important to know that dates are not good for our teeth because dates are rich in sugar and carbohydrates which may give rise to cavities and teeth decay. Dates are a rich source of potassium. Hyperkalemia is the condition where levels of potassium become excessively high in the blood, and consuming too many dates can lead to this condition.

2.4 Spectroscopic determination of metals

2.4.1 Flame atomic absorption spectrophotometry (FAAS)

Flame Atomic Absorption is a very common technique for detecting metals present in samples. The technique is based on the principle that ground state metals absorb light at a specific wavelength. Metal ions in a solution are converted to the atomic state by means of a flame when the light of the correct wavelength is supplied, the amount of light absorbed is measured, and reading for concentration can be obtained. Flame atomic absorption is a very accurate quantitative technique and also a good qualitative technique. This is one of the main reasons for it is the most widely used of the atomic absorption methods. The set up for most flame atomic absorption spectrometers are relatively simple in design.

The most complicated part of the instrument is the nebulizer. The nebulizer is highly important in FAAS. It converts the sample solution into a mist or aerosol. The nebulized sample is then carried into the flame. Radiation from the specific light source is focused on the atomic vapor in the flame.

The radiation then enters a monochromator, which isolates the line of interest and then measured by a photomultiplier tube (detector). The signal is then processed and the computer system prints the output on the screen. Flame atomic absorption spectrophotometry is a sensitive technique for the quantitative determination of metals.

As it is used for determining the concentration of metals it can be applied in environmental analysis and can also be used to detect if there are trace metals present in food (Rihana-Abdallah *et al*., 2015).

2.4.2 Micro plasma atomic emission spectroscopy (MP-AES)

MP-AES consists of a microwave-induced plasma interfaced with an atomic emission spectrophotometer (AES). It is used for the simultaneous multi-analyte determination of major and minor elements. MP-AES employs microwave energy to produce a plasma discharge using nitrogen supplied from a gas cylinder or extracted from ambient air. Samples are typically nebulized prior to interaction with the plasma in MP-AES measurements.

The atomized sample passes through the plasma and electrons are promoted to the excited state. The light emitted as electrons return to the ground state light is separated into a spectrum and the intensity of each emission line measured at the detector. Most commonly determined elements can be measured with a working range of parts per million (ppm) to weight percent (wt.%). MP-AES is a technique comparable to traditional atomic absorption and atomic emission spectroscopy but with several potential advantages including lower cost of operation and elimination of the requirement for flammable gasses (Abed *et al*., 2016).

2.4.3 Refractometry

A refractometer is a simple instrument used for measuring concentrations of aqueous solutions. It requires only few drops of liquid and used as lab equipment throughout the food, agricultural, chemical, and manufacturing industries. When light enters a liquid, it changes direction; this is called refraction. Measuring a refractive index of a liquid is a quick way to evaluate physical and chemical properties such as purity and concentration. The Refractive index is a simple physical phenomenon to find the ratio of light in the speed of vacuum to the speed of light in a medium of interest.

Measuring refractive index involves utilizing Snell's law which relates the light of incidence with the light of refraction and the speed of light in two different mediums or the index of refraction.

$$n_1 \sin\theta_1 = n_2 \sin\theta_2$$

n_1 and n_2 are two different mediums that will impact the refraction.θ_1 is the angle of incidence θ_2 and is the angle of refraction.

Additionally, there are tables to relate the refractive index measured to the traditional standards of sugar concentration. Which is known as the Brix scale. One-degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass. If the solution contains dissolved solids other than pure sucrose, then the °Bx only approximates the dissolved solid content. The °Bx is traditionally used in the wine, sugar, carbonated beverage, fruit juice, maple syrup and honey industries (Etminan, 2020).

The refractive index is affected by three different major factors: The wave length of light, Temperature, and Optical density. The first two factors are typically dependent on the refractometer used. Most refractive index are measured at the yellow doublet D-line of sodium, with a wavelength of 589 nm, as is conventionally done. The final factor affecting refractive index is optical density. A medium of interest with a low optical density, would have also a low refractive index.

2.5 Sample decomposition techniques

There are several types of sample decomposition techniques that are used to convert solid containing samples to solutions suitable for elemental determination by many modern instrumental techniques such as FAAS, ICP-MS, or MP-AES. Sample decomposition for total elemental determination can be applied using digestion techniques which are known as dry ashing, wet digestion, and microwave digestion. The hotplate can also be listed as one of the digestion methods for the measurement of heavy metal concentrations.

2.5.1 Dry ashing

Dry ashing is an alternative means of sample preparation and is accomplished by heating the sample in an open dish or crucible in air. Very often this is done in a muffle furnace located in a cleanroom.

It is mandatory to select an ashing temperature that ensures the quantitative decomposition of organic matter without partial or total loss of analytes by volatilization or by their incorporation into a residue, which is insoluble in usual reagents (Stoeppler, 1992).

2.5.2 Wet digestion

Wet digestion is an important method that involves the chemical decomposition of sample matrices in solution which is usually combined with acids in order to increase solubility.

The various acid and flux treatments are carried out at high temperatures in specially designed vessels that help to minimize contamination of the sample with substances in the air, the local environment, and from the vessel walls (Haynes, 1980).

Acid digestion that is performed using the Kjeldahl apparatus is used to remove the organic constituents that are present in the sample and extract the metals by using concentrated acids.

2.5.3 Hot plate

In laboratory settings, hot plates are generally used to heat glassware or its contents. Some hot plates also contain a magnetic stirrer, allowing the heated liquid to be stirred automatically (Tyagi *et al*., 2008). In an experiment, it can be applied in the digestion of plants or other types of constituents by using different kinds of concentrated acids.

3. Experimental

3.1 Chemicals and reagents

In this study, the chemicals and reagents that were used are all analytical grade. Nitric acid (69-72%) from Sigma Aldrich (Steinleim, Germany) and perchloric acid (70%) from (Aldrich, UK) were used for the digestion of samples. The stock standard solutions containing a 1000 mg/l in 2% HNO_3 of the metals Na, Ca, Mn, Fe, Ni, Cu, Zn, Pb, and Cd (from BDH Chemical Ltd Spectrol, poole England) were used for the preparation of calibration standards of each metal. The glassware and plastic containers that were used in the experiment were washed using a detergent and soaked in dilute nitric acid for 24 hours and then rinsed with deionized water. Deionized water was also used for sample preparation and for the dilution of solutions that were used in the experiment.

3.2 Instrument and apparatus

Round bottomed flask was used in the reflux set up on the Kjeldahl apparatus (Gallenhamp, England) for the digestion of the samples. An oven (J.P. SELECTA, s.a.) was used for drying different types of equipment that were used in the experiment to keep them from dust particles, ceramic mortar and pestle were used to grind the sample to a fine powder. Filter paper (Whatman) was used to separate the filtrate from the residue. Digital analytical balance (Mettler Toledo, Model At250, Switzerland) was used to accurately weigh the mass of the samples. Metal concentration was determined by using flame atomic absorption spectrophotometry (FAAS) (ZEEnit 700p / Analytic jena, Germany).

3.3 Description of the study area

The study areas were selected based on the rank of the world leading countries growing fresh dates and also on the current availability of the samples in Addis Ababa, Ethiopia. These selected places are known to be: Afar, Iraq, and Saudi Arabia which are favorable for the growth of the date palm fruit. Afar is associated with an ethnic people inhabiting the Horn of Africa. They primarily live in the Afar Region of Ethiopia and in northern Djibouti, as well as the entire southern part of Eritrea (Basaloom *et al*., 2019).

Iraq, officially the Republic of Iraq, is a country in Western Asia, bordered by Turkey to the north, Iran to the east, Kuwait to the southeast, Saudi Arabia to the south, Jordan to the southwest, and Syria to the west. The capital, and largest city, is Baghdad (Warda *et al*., 2018).

Saudi Arabia, officially the Kingdom of Saudi Arabia, is a country in Western Asia constituting the bulk of the Arabian Peninsula (Mikhailidis, 2012).

Table 1. The geographical descriptions of the study areas

Sites	Latitude	Longitude	Altitude (m)	Distance from Addis Ababa (Km)
Afar	12^{o}0’0N	41^{o}30’0E	1,600	583
Iraq	33^{o}13’23.5’’N	43^{o}40’45.4’’E	3,550	4,747
Saudi Arabia	23^{o}53’9.4’’N	45^{o}4’45’’E	3,015	5,151

3.4 Sample collection and preparation

The three varieties of date palm fruit samples were purchased from a local market in Addis Ababa, Ethiopia. The date palm fruit samples were stored in a polyethylene bag to keep the samples free of contaminates. Then after the fruit of the date palm was cut, de-seeded and the pulp (flesh) portion was carefully washed using tap water and then rinsed using distilled water. The samples were then open-air dried in the laboratory for 2 weeks to remove as much moisture from the date fruit pulp as possible to inhibit the growth of bacteria, mold, and yeast.

An oven was used to remove the total moisture in the date fruit pulp for 24 hours at a temperature of 105 ^{o}C. Then after the date fruit pulp samples were taken out of the oven and cooled for several minutes. Using a ceramic mortar and pestle it was crushed into powder and then the powder was sieved to separate the fine particles of the date fruit pulp from bigger particles. The finely powdered date fruit pulp samples were then stored in a polyethylene bag for further experimental procedures.

3.5 Sugar content determination of date palm fruit samples

Refractometers measure the degree to which the light changes direction; called the angel of refraction. A refractometer takes the refraction angles and correlates them to refractive index values that have been established. Using this value one can determine the concentrations of solutions.

Date fruit pulp cuts (33 g) were placed in 100 mL of water in a glass beaker and heated using a hotplate (IKARRET, China) at a temperature of 85 ^{o}C kept for 90 min (Chaira *et al*., 2007). The fruits were then separated from the solution using a metal sieve, followed by squeezing the paste and filtering the solution in fine filters.

The solution was passed through a fine metal filter to remove the large fruit tissues followed by finer filtering using cheesecloth wipers. Samples were taken from the solution, cooled for 15 min and the sugar content was determined using J57 Automatic refractometer (Fennir *et al*., 2003). The Rudolph J57 Automatic refractometer is factory calibrated to NIST traceable fluids.

3.6 Analytical procedures

3.6.1 Optimization of digestion procedure

For the digestion of the date palm fruit, different digestion procedures were tested using the mixture of HNO_3, $HClO_4$ by varying the volume of the acid, digestion time, and digestion temperature. An optimized procedure was selected to find the values of these variables that yield the best performance by using a minimum reagent volume, shorter digestion time that gives a clear and colorless solution at lower temperature for the digestion of the date palm fruit. In this study, for the three different samples of the date palm fruit that were used for digestion, the optimum conditions are shown in Tables 2 – 4.

Table 2. Optimization of reagent volume for the digestion of 0.5 g samples of date palm fruit at constant temprature and time

Volume ratio	Temperature	Digestion time	Results
2:1	300	3:00	Colorless and turbid
3:1	**300**	**3:00**	**Clear and colorless solution**
3:2	300	3:00	Colorless with suspension
4:2	300	3:00	Colorless with suspension
5:1	300	3:00	Slightly yellow
6:2	300	3:00	Colorless with suspension

An optimized condition for the digestion of the date palm fruit with the ratio of 3:1 volume HNO_3 to $HClO_4$ at 300^{o}C and 3 hours gave a clear and colorless solution which was preferred as cost minimizing acid ratio.

Table 3. Optimization of temprature for the digesstion of 0.5 g samples of date palm fruit at costant volume and time

Volume ratio	Temperature	Digestion time	Results
3:1	150	3:00	Colorless with suspension
3:1	180	3:00	Colorless with suspension
3:1	210	3:00	Colorless and turbid
3:1	240	3:00	Colorless with suspension
3:1	270	3:00	Colorless and turbid
3:1	**300**	**3:00**	**Clear and colorless solution**

An optimized condition for the digestion of the date palm fruit with a temperature of 300 ^{o}C with a ratio of 3:1 HNO_3 to $HClO_4$ for 3 hours was chosen among all because it has shown a clear and colorless solution.

Table 4. Optimization of time for the digestion of 0.5 g samples of the date palm fruit at constant volume and temprature

Volume ratio	Temperature	Digestion time	Results
3:1	300	30 min	Light yellow
3:1	300	1:30	Slightly yellow
3:1	300	2:00	Colorless with turbid
3:1	300	2:30	Slightly yellow
3:1	**300**	**3:00**	**Clear and colorless solution**

The optimized conditions that gave clear and colorless solutions were found to be 3 mL HNO_3:1 mL $HClO_4$, 300 ^{o}C, and 3 hours, and thus this was selected for digestion of the samples.

3.6.2 Digestion of the date palm fruit

For the determination of metal contents in the date palm fruit the wet digestion method was performed. 0.5 g of powdered and homogenized date palm fruit samples were weighed and transferred to a 250 mL round bottomed flask. Then the mixture of 3 mL HNO_3, 1 mL $HClO_4$ (total of 4 mL) were added based on the optimized procedure followed by fitting the round bottomed flask to the reflux condenser and heating at 300 ^oC for 3 hours until it attains a clear and colorless solution. Without disassembling the flask from the condenser, the solutions which were digested on the Kjeldahl apparatus (Gallenkamp, England) were allowed to cool for 10 min, and also for another 5 min, after removing the condenser. The cooled solution was gently filtered into a 50 mL volumetric flask using Wattman filter paper to remove any suspended matter. The clean solution was diluted up to the mark with deionized water. The blank solutions were also digested in the same way the date palm fruit samples were digested on a triplicate basis.

3.6.3 Calibration of instrument

Table 5. The concentration of working standards, equation of calibration curves, and correlation coefficient

Elements	Wave length (nm)	Concentration of working standards (mg/L)	Equation of calibration	Correlation Coefficient (R^2)
Na	589	1,2,4,8	y = 0.0891x + 0.0276	0.998
Ca	422.7	0.25, 0.5, 1, 2	y = 0.0118x - 0.0036	0.993
Mn	279.5	0.25, 0.5, 1, 2	y = 0.0217x - 0.001	0.999
Fe	248.3	0.25, 0.5, 1, 2	y = 0.0122x - 0.0029	0.992
Ni	232	1, 2, 3, 4	y = 0.0045x + 2E-05	0.996
Cu	324.7	0.25, 0.5, 0.75, 1	y = 0.0118x - 0.0036	0.993
Zn	2139	0.25, 0.5, 0.75, 1	y = 0.051x + 0.0008	0.996
Pb	283.2	1, 2, 3, 4	y = 0.0027x + 0.001	0.998
Cd	213.9	0.25, 0.5, 0.75, 1	y = 0.0273x - 0.0016	0.980

Calibration curves were prepared from standards of known concentration. The curves were established at four concentration levels corresponding to 0.25, 0.5, 0.75 and 1 mg/L for Cu, Zn, Cd, and 0.25, 0.5, 1, 2 mg/L for Ca, Mn, Fe and 1, 2, 3, 4 mg/L for Ni, and Pb. The regression coefficient (R^2) exhibited good linearity and value of 0.99. The calibration curves for each element (Na, Ca, Mn, Fe, Ni, Cu, Zn, Pb, and Cd) are shown in Figure 8 – 16.

After calibration, the sample solutions were aspirated in to the flame atomic absorption spectrophotometer instrument and the metal concentrations were recorded. Three replicate determinations were carried out for each sample. The same analytical procedures were employed for the determination of metals in the blank samples.

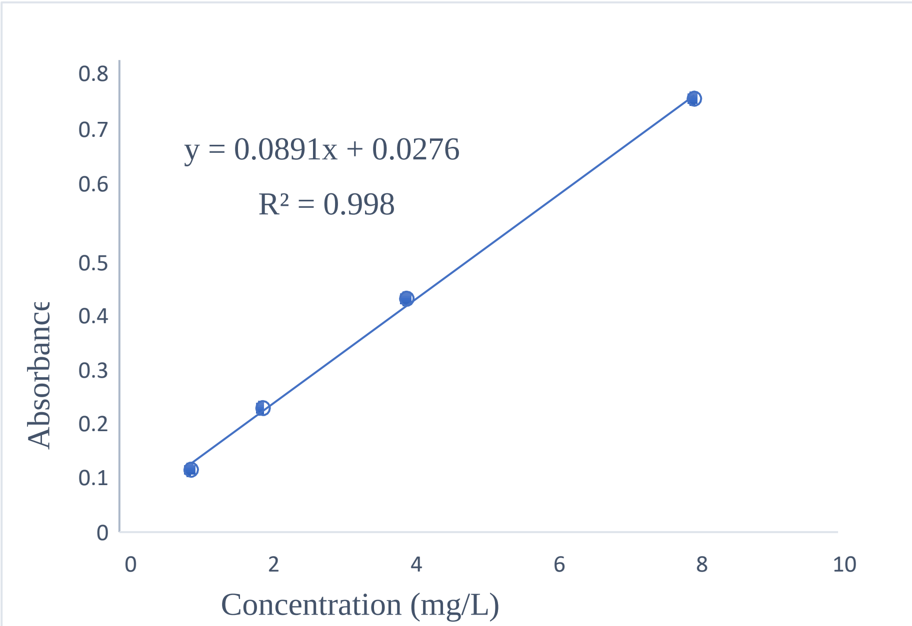

Figure 4. Calibration curve of standard solution of Na

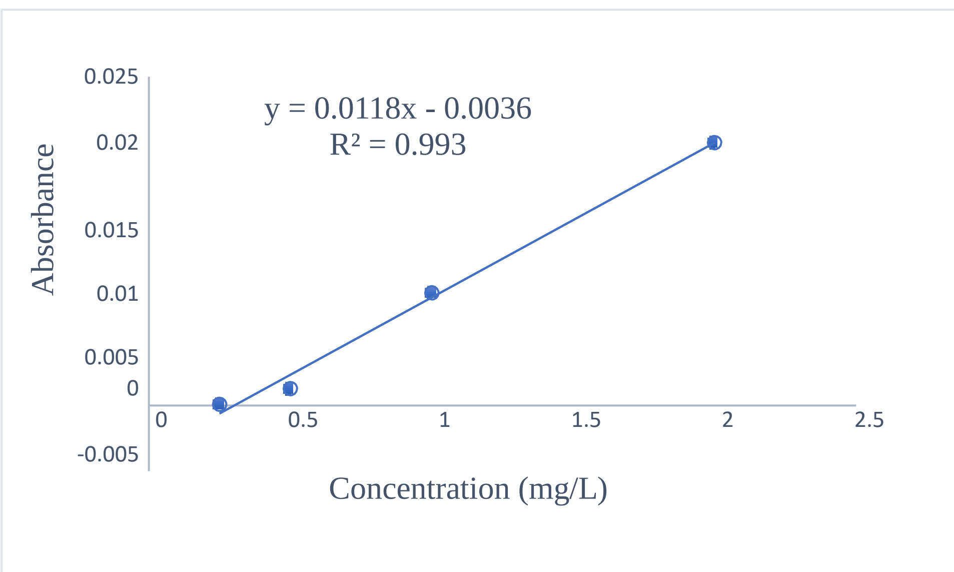

Figure 5. Calibration curve of standard solution of Ca

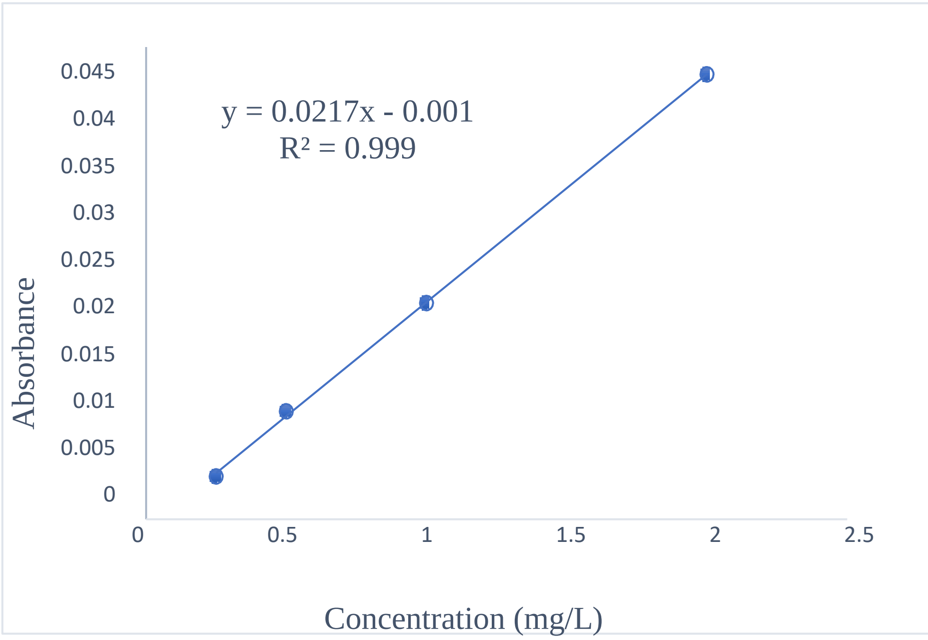

Figure 6. Calibration curve of standard solution of Mn

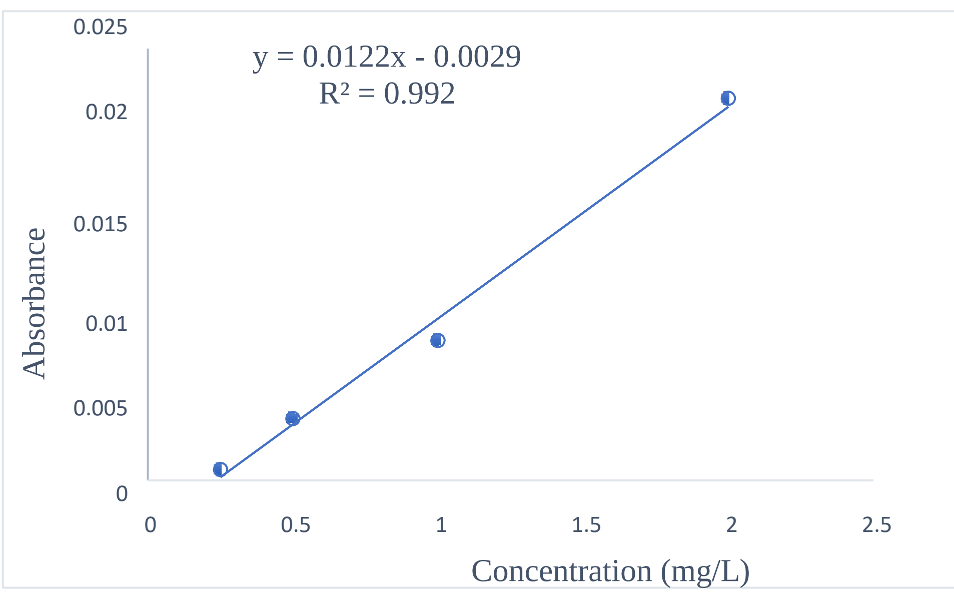

Figure 7. Calibration curve of standard solution of Fe

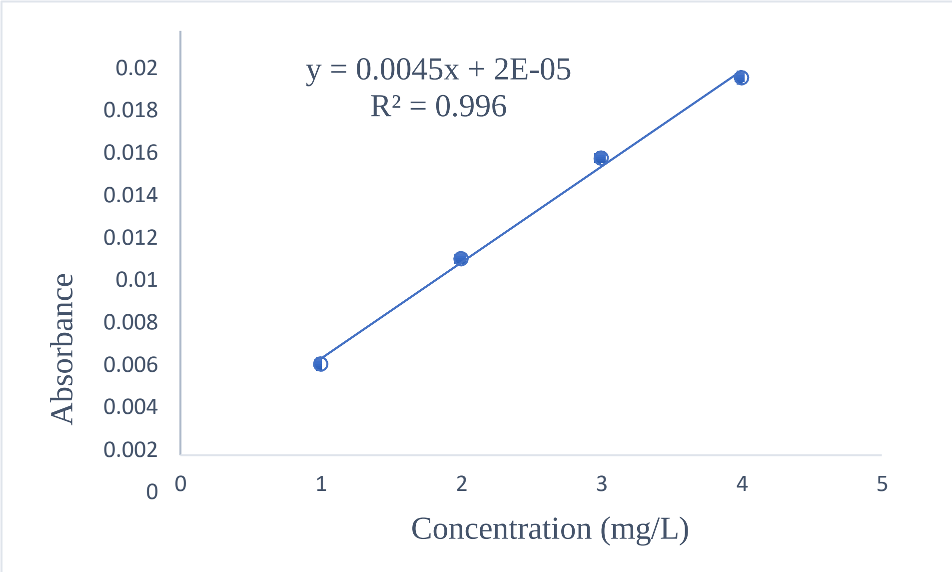

Figure 8. Calibration curve of standard solution of Ni

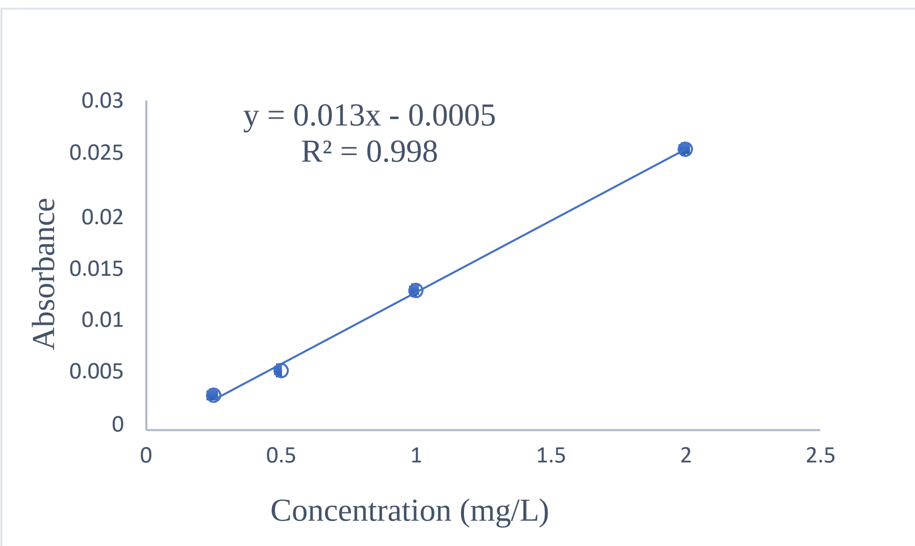

Figure 9. Calibration curve of standard solution of Cu

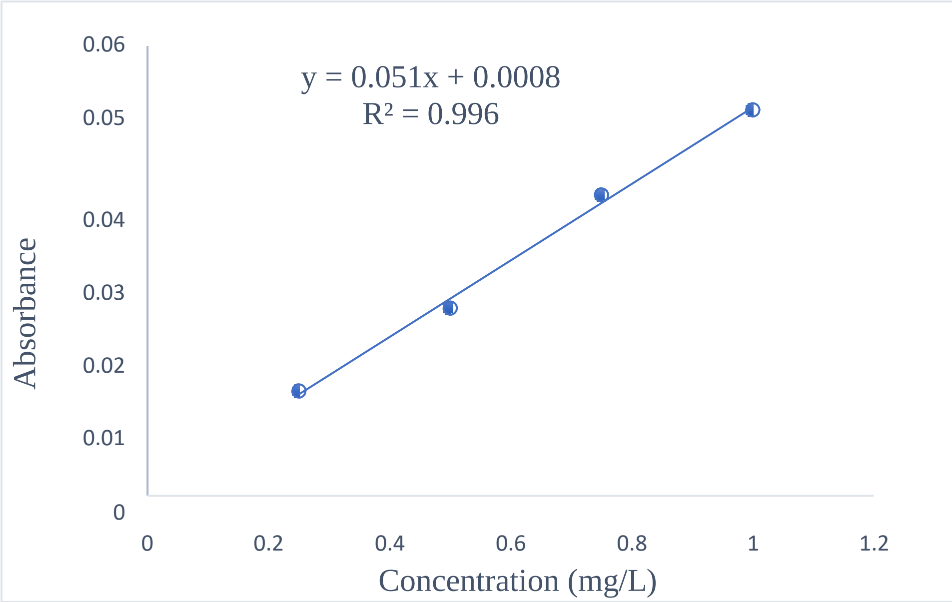

Figure 10. Calibration curve of standard solution of Zn

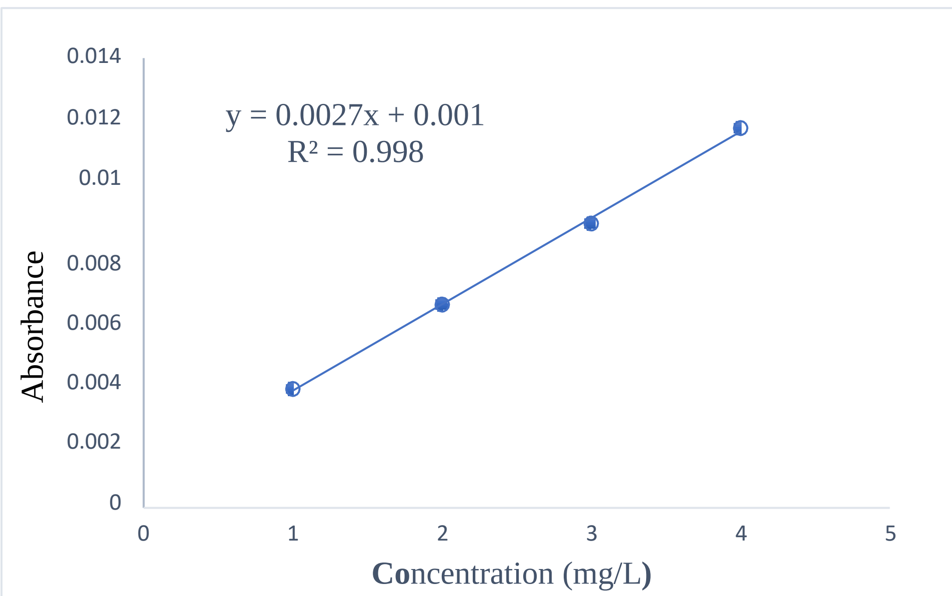

Figure 11. Calibration curve of standard solution of Pb

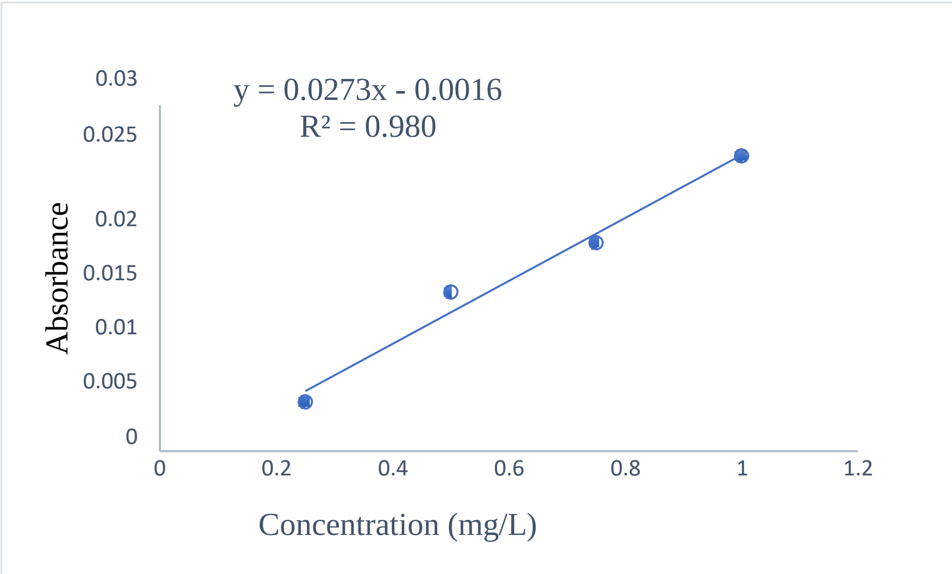

Figure 12. Calibration curve of standard solution of Cd

3.7 Precision

Precision refers to how close the agreement is between two or more measurements. It is determined by a statistical method called standard deviation. Standard deviation is how much, on average, measurements differ from each other. High standard deviations indicate low precision, low standard deviations indicate high precision (Steenkiste *et al*., 2000).

The precision of a measurement is related to reproducibility and repeatability. Repeatability measures the variation in measurements taken by a single instrument or person under the same conditions, while reproducibility measures whether an entire study or experiment can be reproduced in its entirety (Martini *et al*., 2019).

3.8 Method detection and quantification limits

A method's detection limit is the smallest amount or concentration of analyte that can be detected with statistical confidence. The International Union of Pure and Applied Chemistry (IUPAC) defines the detection limit as the smallest concentration or an absolute amount of analyte that has a signal significantly larger than the signal arising from a reagent blank (Goldsmith, 2000). Method detection limit is three times the standard deviation of the blank (i.e. MDL = 3×SD).

The limit of quantification is the smallest concentration or absolute amount of analyte that can be reliably determined. It can be determined as a signal to noise ratio 10:1 or LOQ can be calculated according to the formula: MQL = 10×SD. Where, SD = standard deviation of the blank.

Table 6. Method detection and quantification limits of metals in date palm fruit

Metal	Standard deviation of the blank (SD)	MDL (mg/kg)	MQL (mg/kg)
Na	4.05	12.16	40.5
Ca	0.18	0.55	1.8
Ni	0.27	0.83	2.7
Zn	0.12	0.36	1.2

3.9 Method Validation

Recovery is one of the most commonly used analytical method for validation of analytical results and for evaluating how far the analytical method is acceptable for its intended purpose. Validation of the optimized procedure was performed spiking the samples with standard solutions of known concentration of the analyte elements. Recovery test for the sample was performed in triplicates.

The validity of the digestion procedures was assured by spiking the samples with standard solution of known concentration of the target analytes. The spiked samples were digested and analyzed in similar condition using optimized procedure. As shown in Table 7, four metals were analyzed in triplicate standard metal solutions to evaluate the efficiency of the procedure and percentage recoveries lies within the range from 91.6 % to 97.8 %. The percentage recoveries were calculated using the following equation:

$$\% \text{ Recovery} = \frac{\text{spiked sample} - \text{unspiked sample}}{\text{amount added}} \times 100$$

Table 7. The recovery test result of the optimized procedure for metals in date palm fruit samples

Elements	Concentration of unspiked sample(mg/kg)	Amount added(mg/kg)	Concentration of spiked sample (mg/kg)	% Recovery
Na	9.58	4.79	14.3	97.8
Ca	299	74.7	369	93.7
Fe	115	40.3	152	94
Zn	224	56.0	275	91.6

4. Results and discussion

4.1 Comparison of refractive index values in date palm fruit samples from the different areas

The refractive index values of the three different samples of date palm fruit were analyzed by an automatic refractometer. Among the analyzed samples of the date palm fruit there is a slight difference between the reported refractive index value and measured refractive index values of the date palm fruit samples as shown in the table below.

Table 8. The brix and the refractive index values of three different sample sites

Sample sites	Degree Brix (°Brix) (w/w)	Refractive index reported (De Whalley, 2013)	Refractive index measured (This work)
Afar	14.8	1.35536	1.3554
Iraq	15.2	1.35600	1.3559
Saudi Arabia	15.4	1.35632	1.3563

Among the refractive index values obtained the refractive index value from Saudi Arabia shows a higher index value when it is compared with other sample areas. This indicates the date palm fruit sample from Saudi Arabia have higher sugar concentration because refractive index value increases when the solution gets thicker it results a denser medium which aids the light to be bent and increase its angle of refraction (California State Science Fair Project Listings, 2010).

4.2 Comparison of metals concentration in date palm fruit samples from the different areas

The comparison of metal concentration in date palm fruit samples from the different areas is given in Table 9 and Figures 17-23.

Table 9. Mean concentartions (mean ± SD, n = 3, mg kg^{-1} dry weight) of metals in date palm fruit

Element	Afar		Iraq		Saudi Arabia	
	Mean ± SD	% RSD	Mean ± SD	% RSD	Mean ± SD	% RSD
Na	224 ± 6.50	2.9	320 ± 28.1	8.8	134 ± 9.80	7.3
Ca	299 ± 8.35	2.7	205.5 ± 7.20	3.5	275 ± 6.20	2.25
Ni	25.1 ± 1.59	6.3	28.2 ± 1.94	6.8	42.2 ± 3.87	9.1
Zn	9.58 ± 0.92	9.6	27.9 ± 2.23	7.9	9.27 ± 0.76	8.1
Fe	115 ± 8.53	7.4	114 ± 4.83	4.2	38.8 ± 3.35	8.6
Cu	0.002 ± 0.0001	4.8	0.9 ± 0.04	5.4	1.15 ± 0.02	1.7
Mn	7.11 ± 0.40	5.6	6.66 ± 0.20	3	16.3 ± 0.84	5.1
Pb	ND	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND	ND

ND – Not detected.

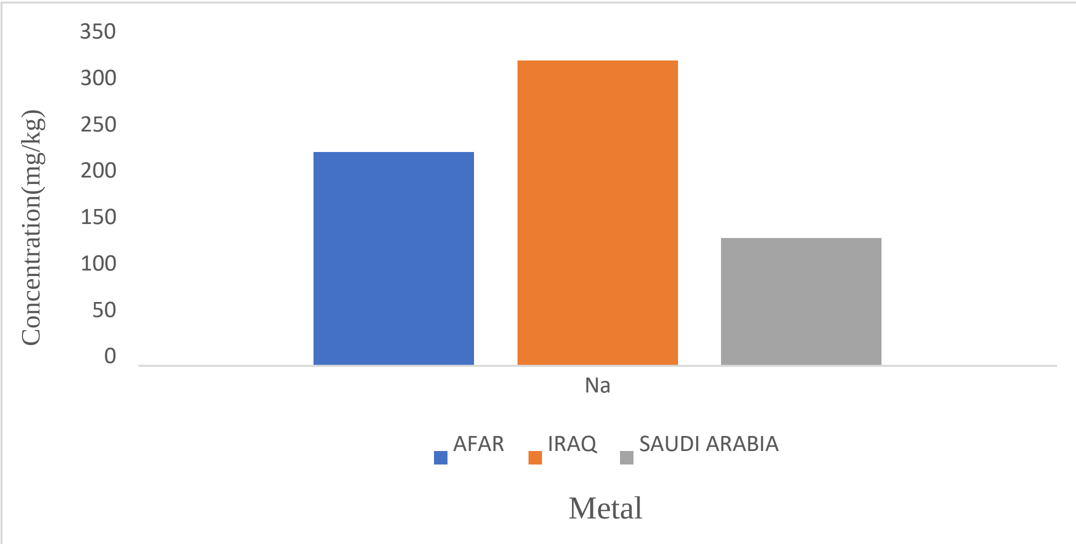

Figure 13. Comparison of Na in date palm fruit from the three different places

As shown in Figure 13, The concentration of Na in Iraq date palm fruit is higher than the concentration of Na in Afar and Saudi Arabia date palm fruit. Also, the mean concentration of Na from Iraq is higher than Afar date palm fruit metal concentration, which is also higher than the Saudi Arabia date palm fruit sample. The mean concentrations of Na in date palm fruit from Iraq, Afar, and Saudi Arabia were 320, 224, 134 mg/kg, respectively.

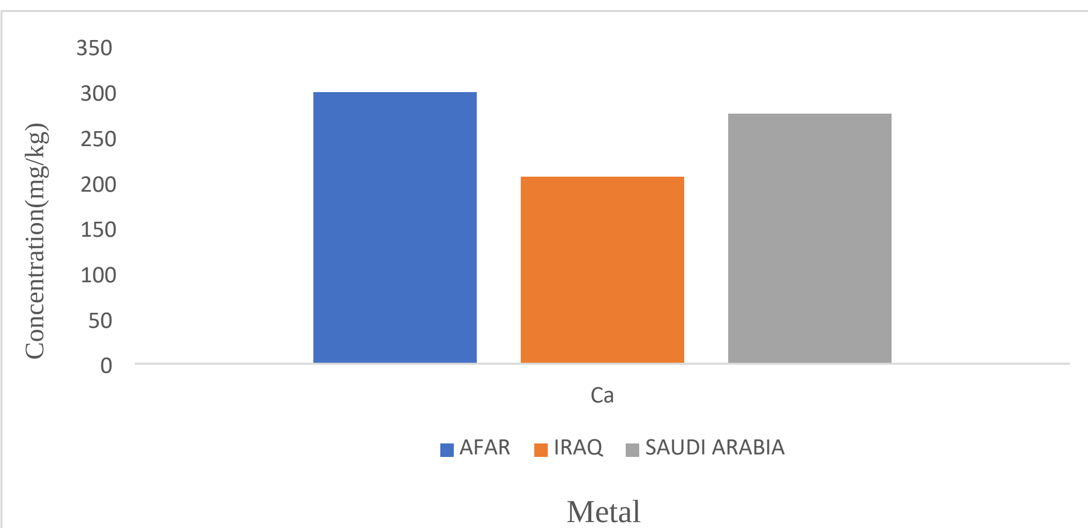

Figure 14. Comparison of Ca in date palm fruit from the three different places

As shown in Figure 14, Ca in the date palm fruit from Afar has a higher concentration of Ca than Saudi Arabia date palm fruit and Iraq date palm fruit. The date palm fruit from Afar has a higher mean concentration of Ca than Saudi Arabia date palm fruit. The concentration in the date palm fruit from Afar, Saudi Arabia and Iraq were 299, 275, 205.5 mg/kg, respectively.

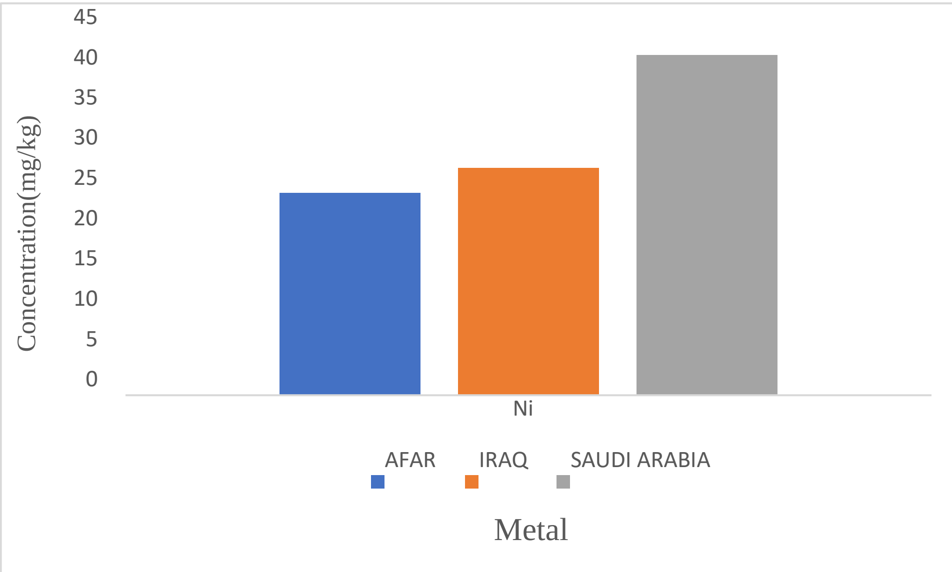

Figure 15. Comparison of Ni in date palm fruit from the three different places

As shown in Figure 15, the date palm fruit samples from Saudi Arabia gave a higher concentration of Ni than the date palm fruit samples from Afar and Iraq. The mean concentration of Ni in Saudi Arabia date palm fruit is higher than Afar date palm fruit mean concentration, which is also higher than Iraq date palm fruit sample. The concentration in the date palm fruit from Saudi Arabia, Iraq and Afar were 42.2, 28.2, 25.1 mg/kg, respectively.

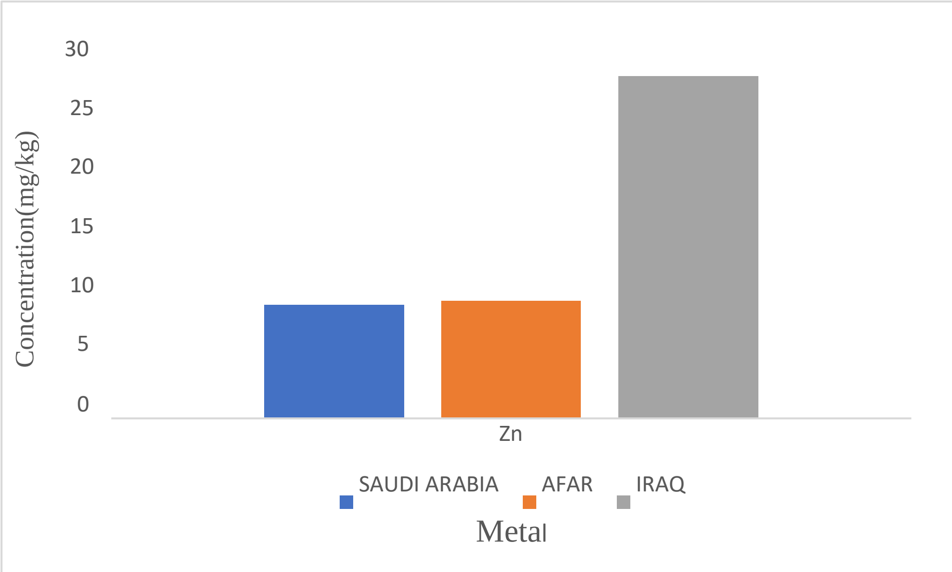

Figure 16. Comparison of Zn in date palm fruit from the three different places

As shown in Figure 16, the concentration of Zn in the date palm fruit sample from Iraq is higher than the concentration of Zn in Afar and Saudi Arabia date palm fruit. The mean concentration of Zn in Iraq date palm fruit is higher than Afar date palm fruit mean concentration, which is also higher than Saudi Arabia date palm fruit sample. The concentration in the date palm fruit from Iraq, Afar and Saudi Arabia were 27.9, 9.58, 9.27 mg/kg, respectively.

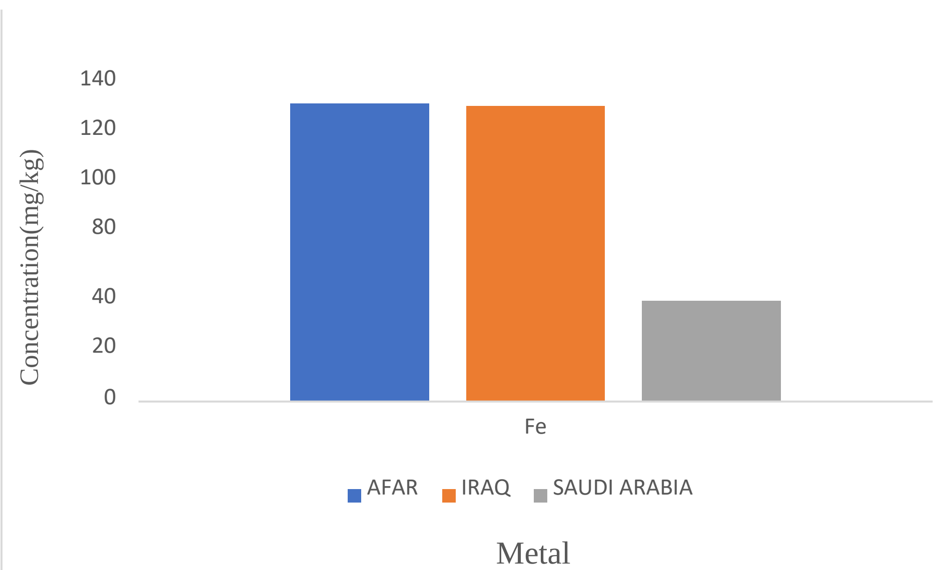

Figure 17. Comparison of Fe in date palm fruit from the three different places

As shown in Figure 17, the concentration Fe is higher in date palm fruit from Afar when compared with date palm fruit from Iraq and Saudi Arabia. The mean concentration of Fe in Afar date palm fruit is higher than Iraq, and Saudi Arabia date palm fruit mean concentration. The concentration in the date palm fruit from Afar, Iraq and Saudi Arabia were 115,114 and 38.8 mg/kg, respectively.

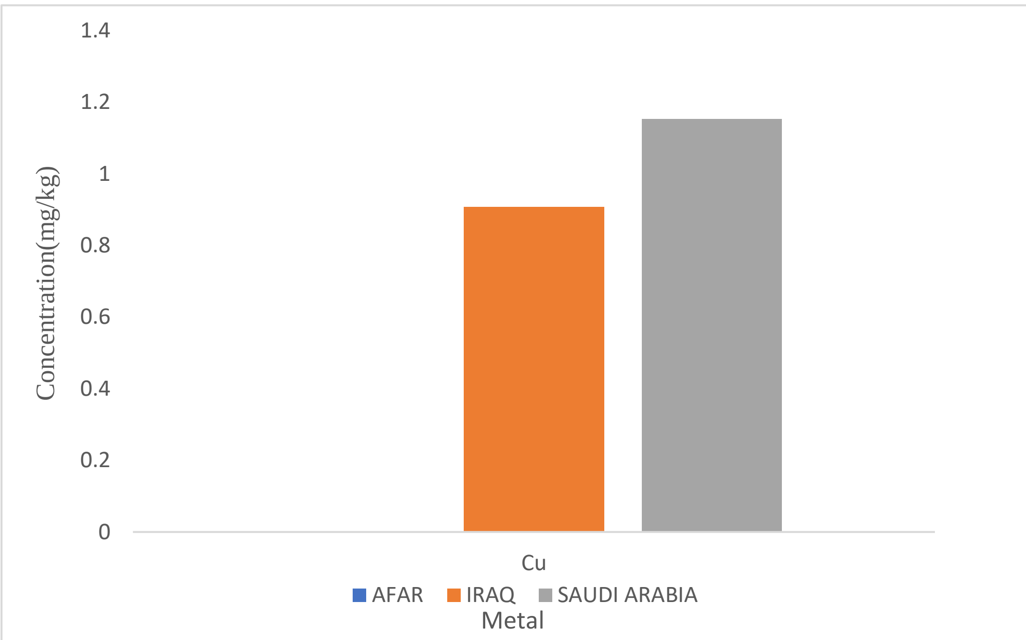

Figure 18. Comparison of Cu in date palm fruit from the three different places.

As shown in Figure 18, The concertation of Cu in Saudi Arabia is relatively higher than the concentration of Cu in Afar and Iraq. The concentration in date palm fruit from Saudi Arabia, Iraq, and Afar were 1.15, 0.90, 0.002 mg/kg, respectively.

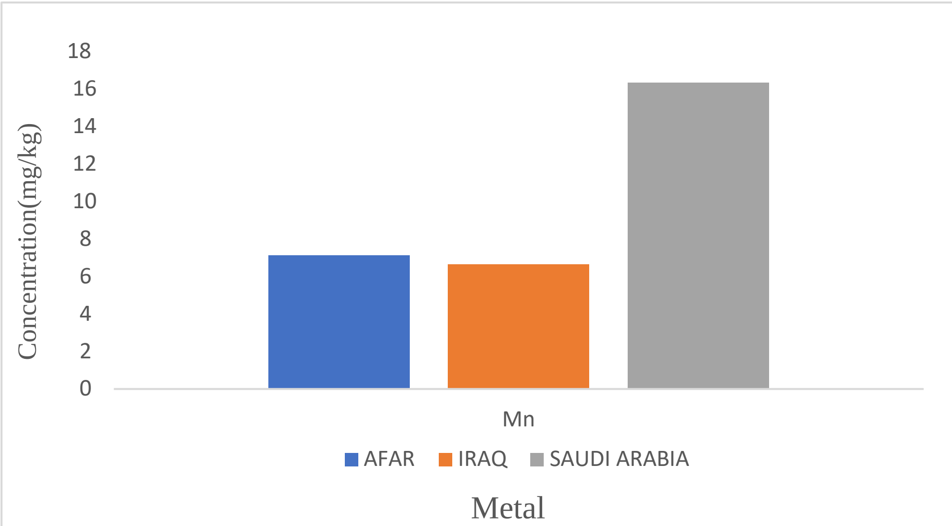

Figure 19. Comparison of Mn in date palm fruit from the three different places

As shown in Figure 19, The concentration of Mn in Saudi Arabia date palm fruit is higher than the concentration found in Afar and Iraq. The concentration in the date palm fruit from Saudi Arabia, Afar, and Iraq were 16.3, 7.11, 6.66 mg/kg, respectively.

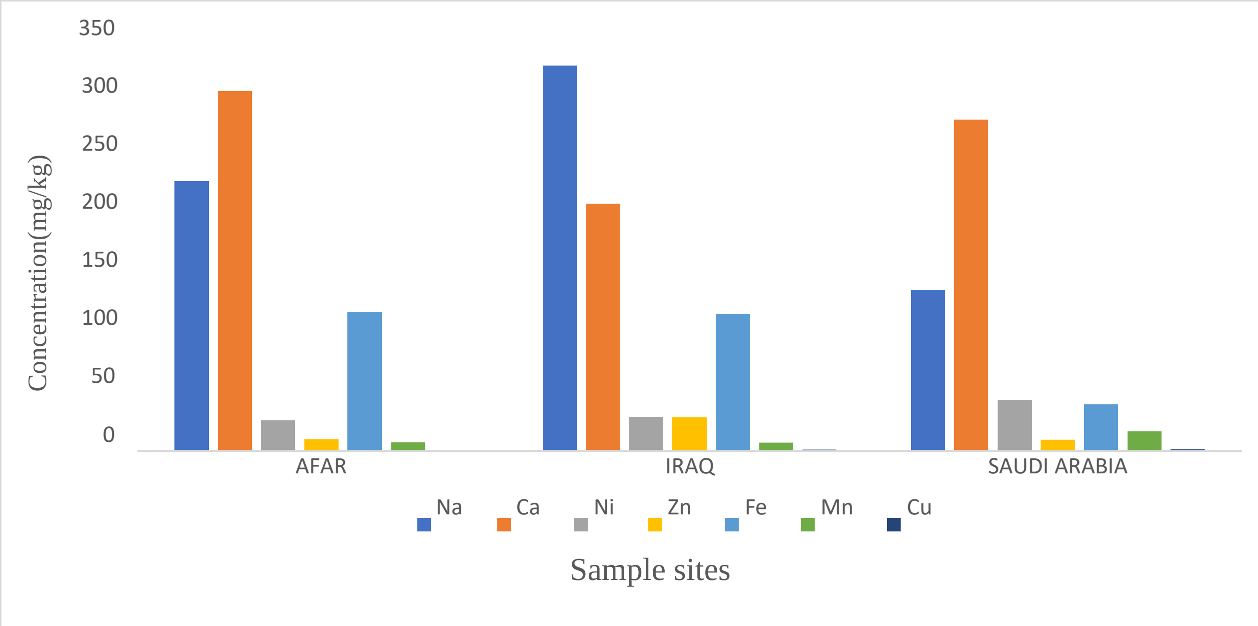

Figure 20. Comparison of Na, Ca, Ni, Zn, Fe, Mn, and Cu contents in the date palm fruit samples from Afar, Iraq and Saudi Arabia.

As shown in Figure 20, the date palm fruit samples from Afar and Iraq contain higher concentration of Na than that of Saudi Arabia. The date palm fruit samples from Saudi Arabia contain higher concentration of Ni than that of Afar and Iraq date palm fruit samples. Similarly, the date palm fruit samples from Iraq contains relatively small amount of Ca. But the Ca content is higher concentration in Afar and Saudi Arabia date palm fruit samples. Fe has relatively a higher concentration in Afar date palm fruit samples when compared with the date palm fruits from Iraq and small amount of Fe is observed in the date palm fruit from Saudi Arabia than that of Afar and Iraq. The concentration of Zn decreased in the trend of Saudi Arabia > Afar > Iraq. Higher concentration of Mn is observed in the date palm fruit from Saudi Arabia compared to Afar and Iraq. In the date palm fruit from Afar the concentration of Cu is very much lower than the date palm fruit samples from Iraq and Saudi Arabia. The mean concentration order of metal content in Afar date palm fruits is Ca > Na > Fe > Ni > Zn > Mn > Cu. For date palm fruits from Iraq the order of metal content is Na > Ca > Fe > Ni > Zn > Mn > Cu and the order of metal content in Saudi Arabia date palm fruit samples is Ca > Na > Ni > Fe > Mn > Zn > Cu.

4.3 Comparison of metal contents in date palm fruit samples with other reported values

Table 10. Comparison of mean concentration of metals in date palm fruit samples with literature reported values

Country	Metal concentration (mg/kg)				Method	Reference
	Na	Ca	Zn	Fe		
Ethiopia	**134-320**	**205.5-299**	**9.27-27.9**	**38.8-115**	**FAAS**	**This study**
Nigeria	912-914	371.65-371.35	16-19	11.50-61.50	FAAS	(Mathew *et al*., 2014)
Malaysia	123-248	488-707	3.28-3.41	8.08-13.48	ICP-OES	(Kalilah *et al*., 2019)
Saudi Arabia	334-338	143-157	0.34-0.45	80.83-202.99	FAAS/ICP-AES	(Kamal *et al*., 2015)
Sudan	566.7-1318.7	222-2381	7.5-10	6.91-69.1	FAAS	(Rania *et al*., 2014)
Pakistan	83.53-102.87	75.39-93.41	-	0.8-0.82	FAAS	(Mohammadzai *et al*., 2010)

Country	Metal concentration (mg/kg)					Method	Reference
	Ni	Cd	Cu	Mn	Pb		
Ethiopia	**25.1-42.2**	**ND**	**0.002-1.15**	**6.66-16.3**	**ND**	**FAAS**	**This study**
Nigeria	-	-	9.84-10.16	10-21	-	FAAS	(Mathew *et al*., 2014)
Malaysia	0.149-0.194	-	4.47-5.04	1.62-3.36	-	ICP-OES	(Khalilah *et al*., 2019)
Saudi Arabia	9.6 -55.64	0.09-28.59	1.15-1.26	53.36-53.94	-	FAAS/ICP-AES	(Kamal *et al*., 2016)
Sudan	-	-	7.1-18.6	5.4-7.8	-	FAAS	(Rania *et al*., 2014)
Pakistan	-	-	-	-	-	FAAS	(Mohammadzai *et al*., 2010)

ND- Not detected

As in Table 10, the ranges of the mean concentrations of the metals determined by flame atomic absorption spectrophotometry method in this study in the date palm fruit samples from Afar, Iraq and Saudi Arabia are presented for comparison with literature values.

The concentration of Na determined in this study ranged from 134 mg/kg (Afar) to 320 mg/kg (Saudi Arabia) which is higher than other reported values except Sudan. The content of Ca determined in this study ranged from 205.5 mg/kg (Afar) to 299 mg/kg (Saudi Arabia) which is relatively higher than other studies except Sudan. The amount of Zn determined in this study ranged from 9.27 mg/kg (Afar) to 27.9 mg/kg (Saudi Arabia) which is higher than other reported values.

The concentration of Fe determined in this study ranged from 38.8 mg/kg (Afar) to 115 mg/kg (Saudi Arabia) which shows comparable results with other reported values except Saudi Arabia. The concentration of Ni determined in this study ranged from 25.1 mg/kg (Afar) to 42.2 mg/kg (Saudi Arabia) which is relatively lower than the other reported values. The concentration of Cu determined in this study ranged from 0.002 mg/kg (Afar) to 1.15 mg/kg (Saudi Arabia) which is relatively higher than other studies except Sudan. The amount of Mn determined in this study ranged from 6.66 mg/kg (Afar) to 16.3 mg/kg (Saudi Arabia) which is higher than other reported studies except Nigeria. The content of Pb, and Cd in this study is below detection limit as also with other reported values.

Table 11. Metal concentrations in date palm fruit (this study) and (RDA/AI) values of metals recommended by agencies and organizations (Whitney *et al*., 2002).

Metals	Mean concentrations of metals (mg/kg)	Amount of metal a person can get from 100 g date palm fruit (mg)	Recommended dietary allowance / Adequate intakes per adult per day (RDA/ AI)
Na	134-320	13-32	1500 mg
Ca	205.5-299	21-30	1000 mg
Fe	38.8-115	4-12	8.0 mg
Zn	9.27-27.9	0.93-2.8	11 mg

The amount of mineral intake by an adult from date palm fruit is as shown in Table 11. The amounts of Na and Ca that an adult can get is well below the recommended values. It indicates that date palm fruits are good source of major metals but not sufficient enough so additional diet is necessary from other sources. The amount of Fe is sufficient. The amount of Zn an adult can get from date palm fruit is below the required amount.

4.4 Statistical analysis

The concept of ANOVA is to compare different sources of variance and make inferences about their relative sizes. In this study one-way ANOVA was used to compare the mean values of the metals between different sample areas. Microsoft Excel was also used for the preparation of calibration curves, and data analysis. The results which were utilized to indicate the absence of significant differences in mean concentration of each metal between the studied date palm fruit samples.

As shown in Table 12, the statistical analysis of ANOVA indicated that there is no significant difference among the mean concentrations of Na, Ca, Fe, Zn, Ni, Mn, and Cu found in the date palm fruit samples from three different areas.

Table 12. Analysis of variance (ANOVA) of date palm fruit samples at 95% confidence level

Metals	F calculated	F critical	Remark
Na	0.04	5.14	No significant difference among the sample means
Ca	0.03	5.14	No significant difference among the sample means
Fe	0.04	5.14	No significant difference among the sample means
Zn	0.01	5.14	No significant difference among the sample means
Ni	0.07	5.14	No significant difference among the sample means
Mn	0.03	5.14	No significant difference among the sample means
Cu	0.007	5.14	No significant difference among the sample means

4.5 Pearson correlation coefficients of metals

Pearson correlation coefficient is a measure of the strength of a linear association between two variables and attempts to draw a line best fit through the data of two variables. The correlation coefficient of experimental analysis indicates how strongly two variables are related to each other. A correlation coefficient of +1.0 indicates a perfect positive correlation, while a correlation coefficient of -1.0 indicates a perfect negative correlation. The correlation values are categorized as no correlation (r = 0.00-0.19), low correlation (r = 0.20-0.39), medium correlation (r = 0.40-0.59), higher correlation (r = 0.60-0.79) and highest correlation (r = 0.80-1.00) (Melina *et al*., 2016). According to the present study highest correlation values were observed between Na-Fe (r = 0.85), Na-Zn (r = 0.88), Ca-Zn (r = -0.96), Fe-Ni (r = -0.98), Na-Mn (r = -0.87), Fe-Mn (r = -0.99). Higher correlation was observed between Na-Ca (r = -0.72), Na-Ni (r = -0.75), Fe-Cu (r = -0.67), Cu-Ni (r = 0.78), Mn-Cu (r = 0.63). Medium correlation was observed between Fe-Zn (r = 0.50), Zn-Mn (r = 0.54), Ca-Cu (r = -0.53), and low correlation was observed in Ca-Fe (r = -0.26), Ni-Zn (r = -0.35),Ca- Mn (r = 0.31), Zn-Cu (r = 0.299). There is no correlation between Ca-Ni (r = 0.10), Na-Cu (r = -0.18).

Table 13. Pearson correlation coefficients of metals in date palm fruit samples

	Na	Ca	Fe	Ni	Zn	Mn	Cu
Na	1						
Ca	-0.72	1					
Fe	0.85	-0.26	1				
Ni	-0.75	0.10	-0.98	1			
Zn	0.88	-0.96	0.50	-0.35	1		
Mn	-0.87	0.31	-0.99	0.97	-0.54	1	
Cu	-0.18	-0.53	-0.67	0.78	0.29	0.63	1

5. Conclusion

Date palm fruit has various advantages due to its tremendous nutritional value. From the results of this study determined by flame atomic absorption spectrophotometry (FAAS) the date palm fruit samples from three different areas contain essential nutrients, such as Ca, Na, Fe, Mn, and Cu. Also, micro essential nutrients such as Ni and Zn were found in the date palm fruit samples. But Pb, and Cd were not detected. The digestion procedure for the determination of metals in date palm fruit samples were optimized and validated through spiking method and good percentage recovery (91.6 % to 97.8 %) were obtained.

The overall mean concentration of the metals (mg/kg) in Afar, Iraq and Saudi Arabia date palm fruit samples collected from the different sampling areas are in the order Ca > Na > Fe > Ni > Zn > Mn > Cu. From the results of this study it is possible to conclude that the date palm fruit samples from three different areas accumulated relatively larger amount of Ca, Na and Fe among the determined metals and lower amount of Ni, Zn, Mn, and Cu. ANOVA indicated that there is no significant differences between the mean concentrations Ca, Na, Fe, Ni, Zn, Mn, and Cu.

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