Quantification of Ca, K, Mg, Zn and Fe elements in grape leaves from different regions of Iraq by atomic absorption spectroscopy

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Abstract. Grapes grow in various regions of Iraq, regions have been selected Baqubah, Al Khalesah, Nasiriyah and Sulaymaniyyah, to determine the Ca, K, Mg, Zn, and Fe concentrations in their leaves for its nutritional importance using atomic absorption spectroscopy (A.A.S.). Two procedures were used for sample preparation (one of their new) and their accuracy data was evaluated, no significant differences at 97% level of confidence were found by comparison the dataset results for two methods. It has been found that Ca, K, Mg, Zn and Fe metal ion concentration are in the range of (806.9 – 2815.2), (7092.2 – 11058.7), (3708.7 – 5434.8), (17.2 – 30.8) and (114.8 - 3083) μg.g-1, respectively and follow the order K>>Mg>Ca>>Fe>Zn. However, the metal contents in vine leaves from Al Khalesah and Baqubah were relatively high in comparing to those of Sulaymaniyyah and Nasiriyah. Fe metal content significantly differs from region to region and the highest concentration was found in grape leaves from Baqubah region (2890.9 - 3083.0 μg.g–1), Nasiriyah (2314.8 - 2331.3 μg.g–1), Al Khalesah (205.7 - 237.2 μg.g–1), while the lowest from Sulaymaniyyah (110.9 - 114.8 μg.g–1). Thus, through the variation in the concentration of trophic metals in grape leaves from one region to another and their lack of similarity, irregularity, and incongruence, it can be concluded that the different concentration of elements depends on the quality of the soil, the irrigation water, and the climate and atmosphere (The nature of environment).

Keywords: Grape Leave, Nutritional Minerals Analysis, Atomic Absorption Spectroscopy, Comparative study.

1. Introduction
Grape leaves are widely used in the cuisines of several cultures, especially in the Mediterranean and Middle East countries. In addition, a rich source of Dietary fiber, a good source of food for providing Ca and vitamin A. About 29 milligrams of Ca and more than 500 international units of vitamin A present in ten grams of grape leaves. Ca is an important element for strengthening of bones and normal tone of muscle while vitamin A is a potent antioxidant that promotes good vision, especially at night. Grape leaves contain less significant amounts of K, Fe, vitamin B-2, vitamin B-3, vitamin B-9, vitamin C and vitamin E, and can provide 7% of the daily value of magnesium, copper, selenium, and manganese [1, 2, and 3]. There are several different types of grapes, the most common of which are V. rotundifolia, V. a Festivalis, V. labrusca, and V. vinifera, for each region specific, but from the 19th century many grape new varieties were grown in different areas of the United States which they were not usually found in other regions. This has led to the globalization of grape species, making it possible to enjoy all varieties in any given region [4 and 5]. The commonly consumed leaves in the Middle East region including Iraq are of cultivated plants (edible and wild) [6]. In addition to good taste, the efficacy of grape leaves can also grow your appetite, so they are important for children growth and development [7]. The antioxidant flavonoids level of grape is high, therefor can have high antioxidant effects. The antioxidants might help to prevent heart disease, blood vessels disease, high blood pressure, and lower density lipoprotein cholesterol (LDL, also called "bad" cholesterol), which then reduces the risk of coronary heart disease and relax blood vessels [8 and 9], in addition to other potentially beneficial effects such as weight loss [10]. Nausea and vomiting caused by chemotherapy [11], Hay fever [12], and Wound healing [13]. For anyone worried about his body weight or diabetics, grape leaves are an excellent choice due to almost no digestible carbohydrates, sugar and contain virtually no fat. For example, it has less than 30 calories, which is probably less energy than it takes for you to chew, swallow and digest them. And due to the lack of sugar, it has an incredibly low glycemic index of zero nearly at eating grape leaves. Also they are supported by high dietary fiber that can lower the blood glucose level [14]. Biomolecules in the extract of grape leaves are involved in the one-step synthesis of iron-based nanoparticles [15]. It turns out, whole grape is a wonderful antioxidants source, but grape leaves are rich in antimicrobial and antioxidant compounds as well. The piece of a research published in IJOFP in early 2013 compared properties of the antioxidant of grape leaves, which collected in May, August, and September. It found that the grape leaves plucked in September has the highest levels of strong properties for antioxidant (total phenols, flavonoids, flavanols, and stilbenes). It was found that grape leaves extract possesses antimicrobial activity against Escherichia coli, Salmonella Infant is, Staphylococcus aureus, Bacillus cereus, and Campylobacter [16]. Grape leaves dry has been used for many centuries in the Mid-east region as herbal medicine to combat a variety of cases including heavy menstrual bleeding, diarrhea, liver inflammation, stomach aches, and arthritis. Also, they are used to treat ulcers, toothache, gingivitis, and the problem of bad breath, pain and problems with urination, grape leaves extract findings to suggest effective cytotoxic activity against lung cancer cells, anticancer and antimicrobial activities [17]. Grape leaves contain mildly anti-inflammatory compounds that can reduce edema. That is especially important for people with lower legs chronic vein problems. The grape leaves plant extracts to reduce edema in patients with chronic venous insufficiency, according to a research. Problems of veins returning blood in the legs back to the heart that is a condition characterized when blood cannot return to the heart, the legs will be swelling causing edema. A reduction of edema will not only make the individual more comfortable by reducing the swelling, so it will cause less of a strain on the vascular system [18]. A study showed that grape leaves may protect the brain from memory loss, possibly aiding in Alzheimer's
prevention. Another study was examined on the potential therapeutic effects of grape leaves’ water extract and found it may help reduce inflammation and protect the liver. The study highlights the potential for improvement of the liver damage of hepatic fibrosis, or scarring of the liver, because of damage. The study also showed grape leaves’ ability to reduce oxidative stress and associated inflammation [19]. A diet high in potassium can lead to reduced blood pressure levels and positive effects on heart health, stroke, anxiety, and stress, and enhance muscle strength and water balance in the body [20 and 21]. A diet of calcium, for as our body needs calcium to build and maintain strong bones, our heart, muscles, and nerves also need calcium to function properly. Some studies suggest that calcium, along with vitamin D, may have benefits beyond bone health, perhaps protecting against cancer, diabetes, and high blood pressure, but evidence about such health benefits is not definitive [22]. While a magnesium diet, is a cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation, a trace amount is only needed for the human body [23 and 24]. As for a diet of iron, iron has many important functions in our bodies, it is necessary for the transport of oxygen in red blood cells [25 and 26]. Zn is an essential mineral that we need to stay healthy. It aids growth, DNA synthesis, immune function and more, role in our bodies, it is a vital mineral that your body uses in countless ways, it is necessary for the activity of over 300 enzymes that aid in metabolism, digestion, nerve function, and many other processes, it is also fundamental to skin health, DNA synthesis and protein production, What’s more, body growth and development relies on zinc because of its role in cell growth and division, it is also needed for our senses of taste and smell, because one of the enzymes crucial for proper taste and the smell is dependent on this nutrient, a zinc deficiency can reduce our ability to taste or smell [25]. There are many mineral elements including potassium, calcium, magnesium, iron, and zinc are known to be essential to have a healthy life of humans. The literature data stated that grape leaves are a good source of them. However, the metal concentrations in the grape leaves depend on their availability to uptake by the vine plant which depends on example soil, pH, climate, and water source etc. Therefore, we decided to investigate their contents in these leaves depending on grown in different regions in Iraq namely. Because of the importance of these elements and their nutritional benefits, we considered their quantitative estimate for a distinction between grape leaves of different regions.
The aim of this research is studies to determine the metal ion concentrations (Ca, K, Mg, Zn, and Fe) on the nutritional value of grape leaves according to geographical location.

2. Materials and methods
2.1 Experimental Details and Conditions
The atomic absorption spectroscopy, model Phoenix-986 AA (UK), is used with a graphite furnace attachment for flameless analysis. It is fitted with a motorized Flame/Furnace switchover. The automatic switchover between the flame and graphite furnace analysis modes is done by a keystroke. It is supplied with automatic temperature control and a motorized multi-element analysis with eight lamp turret which allows the automatic positioning and optimization of each H.C.L. (Hollow Cathode Lamp) by the software. The spectral bandwidth is automated and available with a choice of five slit sizes (0.1nm, 0.2nm, 0.5nm, 1.0nm and 2.0nm). A hydride generator was used for a quantitative determination of Ca, K, Mg, Zn, and Fe ion contents at an ultra-low level. The hydride generator is supplied with an absorption cell, electrical absorption cell heater, controller, and all necessary burner fittings. We offer a low-cost
alternative where no efforts were spared to provide high precision and ease of use in a simple instrument which can fit in every budget. This method describes the determination of Calcium in fertilizers by atomic absorption. The less-sensitive wavelength of 422.7 nm is used to avoid excessive dilution, determination of Potassium at 404.4 nm, determination of Magnesium at 285.2 nm, determination of Zinc at 213.9 nm and determination of iron at 248.3 nm. The samples were weighed using an Ohaus Adventurer Pro AV264 analytical balance (Made in Switzerland) with a resolution of 0.1 mg and tare maximum of 260g. For the next step of sample acid digestion.

2.2 Collection of plant Leaves
Fresh plant leaves of grape were collected from four different regions in Iraq namely, Sulaymaniyyah, Nasiriyah, Al Khaleesah and Baqubah, as shown on the map of Iraq in Fig.1. A total of four samples of grape leaves (10 healthy and completely) were collected from four different grape plants of the same variety. The grape leaves were cleaned by washing several times with deionized water, dried through dry air then crushed to powder using grinding machine. The powders were storage at 15 °C in sealed bottle.

2.3 Standard Solution Preparation
For calibration curves of metal ions in A.A.S., standard solutions (from the device manufacturer) were prepared by diluting 1000 mg/L standard solutions with deionized water to obtain 10 - 100 mg/L of calcium, potassium, and magnesium, and 1.0 – 10.0 mg/L of iron and zinc. Then by using an autosampler to adjust the injection volumes of standard solutions in a stepwise manner calibration curves were generated.

2.4 Sample Solution Preparation
Two procedures were used for sample preparation as follow:

2.4.1. (a) A weighed amount of grape leave powder (1.0 - 0.5 g) was placed in a digestion vessel. Then, 10 mL of HCl (6N) were added to the powder, and the mixture heated at (≤50˚C) for 24 hours on hot plate (Thermostat at 45˚C). After the digestion, the samples were transferred to volumetric flask (25mL) and diluted with deionized water to a specific volume. The addition of HCl may limit the techniques or increase the difficulties of analysis, but dissolved these metal ions in plant tissue, and eliminates elemental loss by volatilization because the digestion takes place at a low temperature (New procedure).

2.4.2. (b) A weighed amount of grape leave powder (1.0 - 0.5 g) is placed in a digestion vessel, followed by addition mixture of 10 mL of concentrated HNO3 and 2mL of H2O2. The mixture was heated for 10 minutes in microwave for digestion. After that, the samples were diluted to a specific volume (25mL) with deionized water [27].

3. Results and Discussion

The concentration of selected the metals in a grape leave following procedure (a) and following procedure (b) are shown in Table.1 and Table.2, respectively. In addition to the sample preparation of grape leaves, the data compared for accuracy and precision (metal analysis measurement for six sample solutions of grape leaves per each city).

**Table 1. Concentrations of the selected metals (mg/L) in 25 mL of sample (procedure a).**

| No. | Area of Iraq | Mass of Grape Leave (g)* | Ca ppm | K ppm | Mg ppm | Zn ppm | Fe ppm |
|-----|--------------|--------------------------|--------|-------|--------|--------|--------|
| 1   | Baqubah      | 0.6325                   | 26.5   | 184   | 137.5  | 0.58   | 78     |
| 2   | Al Khalesah  | 0.7415                   | 83.5   | 328   | 110    | 0.82   | 6.1    |
| 3   | Nasiriyah    | 0.5076                   | 22.5   | 144   | 100    | 0.35   | 47     |
| 4   | Sulaymaniyah | 0.7622                   | 24.6   | 312   | 125    | 0.94   | 3.5    |

*Six sample solutions average of mass of grape leaves (g), a hot-plate acid-digested at (≤50˚C) in 25 mL.

**Table 2. Concentrations of the selected metals (mg/L) in 25 mL of sample (procedure b).**

| No. | Area of Iraq | Mass of Grape Leave (g)* | Ca ppm | K ppm | Mg ppm | Zn ppm | Fe ppm |
|-----|--------------|--------------------------|--------|-------|--------|--------|--------|
| 1   | Baqubah      | 0.6875                   | 28.7   | 198.4 | 147.5  | 0.62   | 79.5   |
| 2   | Al Khalesah  | 0.5165                   | 58.4   | 228.4 | 77.9   | 0.58   | 4.9    |
| 3   | Nasiriyah    | 0.6863                   | 29.7   | 192.6 | 136.1  | 0.48   | 64     |
| 4   | Sulaymaniyah | 0.6538                   | 21.1   | 271   | 106.5  | 0.76   | 2.9    |

* Six sample solutions average of mass of grape leaves (g), a microwave acid-digested in 25 mL.

Metals analysis measurement of four sample solutions comprising of grape leaves that are regularly consumed. These samples from Baqubah, Al Khalesah, Nasiriyah and Sulaymaniyah in Iraq. All the
elements were detected in grape leaves sample solutions. Calculation of quantitation elements as a concentration in grape leaves powders (μg/g) are shown in Table.3 for the procedure (a) and Table.4 for the procedure (b).

Table 3. Concentrations of the selected metals (μg/g) of grape leaves sample (procedure a).

| No. | Area of Iraq | Ca (µg/g) | K (µg/g) | Mg (µg/g) | Zn (µg/g) | Fe (µg/g) |
|-----|--------------|-----------|----------|-----------|-----------|-----------|
| 1   | Baqubah      | 1047.4    | 7272.7   | 5434.8    | 22.9      | 3083.0    |
| 2   | Al Khalesah  | 2815.2    | 11058.7  | 3708.7    | 27.6      | 205.7     |
| 3   | Nasiriyah    | 1108.2    | 7092.2   | 4925.1    | 17.2      | 2314.8    |
| 4   | Sulaymaniyah | 806.9     | 10233.5  | 4100      | 30.8      | 114.8     |

Table 4: Concentrations of the selected metals (µg/g) of grape leaves sample (procedure b).

| No. | Area of Iraq | Ca (µg/g) | K (µg/g) | Mg (µg/g) | Zn (µg/g) | Fe (µg/g) |
|-----|--------------|-----------|----------|-----------|-----------|-----------|
| 1   | Baqubah      | 1043.6    | 7214.5   | 5363.6    | 22.5      | 2890.9    |
| 2   | Al Khalesah  | 2826.7    | 11055.2  | 3770.6    | 28.1      | 237.2     |
| 3   | Nasiriyah    | 1081.9    | 7015.9   | 4957.7    | 17.5      | 2331.3    |
| 4   | Sulaymaniyah | 806.8     | 10362.5  | 4072.3    | 29.1      | 110.9     |

An Atomic Absorption Spectrophotometry (AAS) was used for the determination of Ca, K, Mg, Zn and Fe as shown in the experimental section. An acid digestion procedure can be used for sample preparation in the determination of many elements in plant tissue, including Calcium, Potassium, Magnesium, Zinc, and Iron. A weighed amount of dried grape leaves was placed in a digestion vessel where, acid was added, and the mixture was heated on a hotplate or in a microwave to complete digestion. After the digestion, the samples were diluted to a specific volume.

The grape leaves solution aspirating in atomic absorption spectroscopy then converting the digested sample into a liquid/gas aerosol, the treated sample into a characteristic flame. The main advantage of this method is that it eliminates elemental loss by volatilization because the digestion takes place at a temperature (≤50°C) or microwave, unlike previous methods that required heating to boiling point to digest the sample. But the main disadvantages of this method are that it is subject to reagent contamination (acid) and requires operator attention only.

By using two procedures of sample preparation, no significant differences from the comparison between the results at the 97% confidence level were found. The results of this study indicate that different Ca, K, Mg, Zn and Fe concentrations are present in grape leaves depending on their local grow. We found that Potassium and Magnesium concentrations are higher than 1000 µg/g followed by Calcium, Iron and Zinc (lower than 10 – 1000 µg/g). Potassium concentration level is higher than Magnesium, Calcium, Iron and Zinc (K>>Mg>>Ca>>Fe>Zn), as shown in Chart.1 for the procedure (a) and Chart.2 for the procedure (b).
The concentrations of the metals in samples from Al Khalesah and Baqubah were found to be relatively higher than other samples from Nasiriyah and Sulaymaniyah. Also, it can be noticed that there are significant differences in potassium concentration in almost all of the regions from (11058.7 μg.g⁻¹) in Al Khalesah to (7092 μg.g⁻¹) in Nasiriyah.

It has been found that grape leaves from Al Khalesah were higher concentration of Calcium and Potassium than other samples, while the concentration of Magnesium and Iron in grape leaves from Baqubah were higher than other samples, and the concentration of Zinc in grape leaves from Sulaymaniyah higher than other samples.

Diagrams as shown in Chart 1 for the procedure (a) and Chart 2 for the procedure (b), the mineral content of Fe vary significantly from region to region, and the highest concentration of grape leaves was found in Baqubah, but a K concentration remains at the highest amount among the food minerals under study, while the amount of Mg remains almost fixed.

Chart 1. Amounts of metals in grape leaves (procedure a).

Chart 2. Amounts of metals in grape leaves (procedure b).
Acknowledgment
Grape leaves are an important dietary source of K, Mg, Ca, Fe and Zn elements. The grape region influences the concentration of five major metals, Zn (has the lowest concentration) and K (has a higher concentration). Metals analysis of these samples found that, a sample from Baqubah were a higher concentration of Mg and Fe than other samples, a sample from Al Khalesah, were higher concentration of K and Ca than other samples, and a sample from Sulaymaniyah were higher concentration of Zn than other samples. Thus, it can be concluded that the concentrations of elements in the leaves of grape are influenced by the region depend on soil type and irrigation water [28].

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