An Experimental Study of the Effects of the Mud Boil-up Process on the Physico-Chemical Properties of Grape Syrup

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ABSTRACT: *The deficiency of calcium, magnesium, potassium, and iron leads to severe disorders in human body organs. This study introduces Dooshab as a food source of these useful elements. The Dooshab which translates to grape syrup is widely used in Iran. In the traditional method of the Dooshab production, the white soil is added to the grape juice and the mixture is boiled. After cooling, it is filtered. To get the syrup, the filtered juice is boiled again and concentrated. In this research grape juice was divided into two equal parts. A known amount of white soil was added to one of the samples. Then, both samples were boiled and left to cool down. After paper filtering, the pH of each sample was measured. Heating was continued until liquids were concentrated and converted to syrup. Both samples which are produced from using mud-boiled up stage and skipping this step were diluted to fixed volumes. Similar amounts of two samples were ashed and dissolved in distilled water and tested. The atomic absorption spectroscopy, spectrophotometry, and flame atomic emission spectroscopy techniques were used for quantitative analyses. The calcium, magnesium, iron and potassium quantity are 12 mg, 6.82 mg, 2.32 mg, and 250 mg in 25 mL of the Dooshab and 1.75 mg, 0.65 mg, 1.28 mg, and 65 mg in 25 mL of grape sauce, respectively. This study demonstrates that boiling of grape juice with the white soil decreases the acidity and increases the amounts of useful elements in grape syrup.*

KEYWORDS: *Dooshab; Grape syrup; Grape sauce; Spectroscopy; Quantitative analysis*.

INTRODUCTION

Calcium, magnesium, potassium, and iron are four elements that play important roles in the human body. Calcium is the most abundant mineral in the body. About 99% of the body's calcium is found in bones and teeth. The remainder is present in intercellular fluid and serum. Almost half of calcium is ionized and biologically active. It is also essential for other biological actions including blood coagulation, heart and skeletal muscles stimulation, neural impulses conduction, and setting of endocrine and exocrine glands [1].

Magnesium is one of the most important intercellular cations in the body. Approximately, all of the magnesium

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is stored in soft tissues, muscles, and bones and just 1% of total magnesium is in serum and extracellular fluid. Every organ in the body, especially the heart, muscles, and kidneys, needs magnesium. This mineral also contributes to the make-up of teeth and bones. Magnesium activates enzymes, contributes to energy production, and helps regulate levels of calcium, copper, zinc, potassium, vitamin D, and other important nutrients in the body. Hypomagnesemia may cause hypocalcemia and a broad array of neuromuscular, cardiovascular, or respiratory symptoms. Commonly, this occurs in an Intensive Care Unit (ICU) setting in which magnesium administration and diet are inadequate [1].

Potassium is a very important mineral for the proper function of all cells, tissues, and organs in the human body. It is also an electrolyte, a substance that conducts electricity in the body, along with sodium, chloride, calcium, and magnesium. Potassium is crucial to heart function and plays a key role in skeletal and smooth muscle contraction, making it important for normal digestive and muscular function [2].

Iron is an essential element for blood production. About 70% of human body's iron is found in the red blood cells of blood called hemoglobin and in muscle cells called myoglobin. Hemoglobin is essential for transferring oxygen in the blood from the lungs to the tissues. Myoglobin, in muscle cells, accepts, stores, transports, and releases oxygen. About 6% of body's iron is a component of certain proteins, essential for respiration and energy metabolism, and as a component of enzymes involved in the synthesis of collagen and some neurotransmitters. Iron also is needed for proper immune function. When iron stores are exhausted, the condition is called iron depletion. Further decreases may be called iron-deficient erythropoiesis and still further decreases produce iron deficiency anemia [2].

If the body does not take these elements from diet enough, deficiency of them occurs and leads to some disorders in organs. In the present study, a natural source for calcium, magnesium, potassium, and iron is introduced. The Dooshab (i.e., the grape syrup(1)), like honey, is widely used in Iran. Physicians advise people with iron deficiency anemia and arthritis to use grape syrup. In the traditional method of producing grape syrup, when the grape juice is produced, some white soil is added and the mixture is boiled. It is then cooled and filtered. Thereafter, the filtered liquid is further boiled and concentrated to obtain the syrup. If the soil is not added and a so-called "mud boil-up" process is not done, the syrup appears turbid and brown (not red) and does not taste sweet. From the health point of view, it is recommended that people with low iron deficiency anemia or arthritis may use Dooshab and hyperkalemia(2) patients may not use it (Personal Communications with Dr. *S.M. Hashemi Dehkordi*, Department of Food Industries, Islamic Azad University, Shahrekord, Iran, 2016). The white soil contains calcium, iron, potassium, and magnesium. It is expected that "mud boil-up" process influences the amounts of these elements and properties of syrup [3].

In this research, two syrup samples, one obtained without adding the white soil (the product is grape sauce) and the other produced with adding the white soil (the product is Dooshab) were studied. Their appearance, acidity, and amount of iron, calcium, magnesium, and potassium were also measured.

Properties of the Grape Products

Grape as fresh fruit and its products such as juice, raisins, syrup, and seeds contain bioactive compounds with high antioxidant capacities [4, 5].

The grape juice and its concentrated products contain high amounts of phytochemical compounds. Researches on human showed that the use of violet grape causes a decrease in blood pressure and Alzheimer's disease [6]. Studies also showed that a rich grape diet have wide effect in heart disease [7].

Studies showed that regular use of grape syrup in the diet increases the total antioxidant capacity in plasma, high density lipoprotein, fibrinolytic, and anti-thrombin activity. From the nutritional value perspective, the grape syrup is very rich and nourishing and most of its efficiency is in brain operation, thus it is useful for kids and athletes. It is also anti-flatulent and causes ejectment of urea from the body [8].

The Dooshab is a whole natural nutrition-rich food that contains glucose and fructose sugars, minerals, organic acids, and considerable amounts of Vitamin A, B,

(1) The Dooshab and grape syrup are interchangeably used in this text.

(2) An elevated concentration of the electrolyte potassium (K^+) in the blood

and C [8, 9]. The Dooshab contains 2.93 calories per gram [10]. Recent researches showed that the grape syrup is a good natural substitute for sugar in the confectionery industry as production of Halva and Gaz [11, 12]. It is also used as a natural coloring and taste improver in bakery, dairy and beverage industry [10, 13-16].

EXPERIMENTAL SECTION

All chemicals used in our analysis were of analytical reagent grade (Merck) and used without further purification and in all of the steps distilled water was used. The apparatuses are a S 2100 UV Spectrophotometer for the absorption measurements, a Varian 220 Model for the atomic absorption measurements, a Fater Electronic Model Flame Photometer for the atomic emission measurements and a HORIBA M-12 pH meter for pH determination.

Preparation of samples

Exactly 4 kg of the grape used for producing the Dooshab was washed and rinsed with distilled water. Then, it was granulated and juiced. The juice was divided into two parts (1550 g each) using two numbered beakers. 50.00 g of the white soil was added to one of the beakers [3]. The beakers were heated to boiling point then left to cool down. After filtering by filter paper, pH of each sample was measured and then heating was continued until liquids were concentrated and converted to syrup. Each sample was transferred into a 250-mL flask and diluted to the mark with distilled water. The sample with added soil was marked as number 1 and the other was labeled number 2. Exactly 25.0 mL of each sample were transferred into the numbered porcelain crucibles. Each sample was then heated to dryness on an electric heater. The heating was continued until fume has come out. The process was stopped at this point until a black solid was seen. The residues were ashed in furnace at 900°C. After cooling, 6.0 ml of concentrated nitric acid was added to each sample and they were diluted to 100 ml in volumetric flasks.

Acidity of samples

The measured pH was 4.85 for sample 1 and 3.44 for Sample 2.

Determination of Iron by Absorption Spectrophotometry Method, Plotting Standard Addition Curve

In five 25-ml numbered volumetric flasks, 0.0, 0.5, 1.0, 1.5, and 2.0 mL standard Fe^{3+} solution (20 ppm[(1)]) was added, respectively. In each flask, 1.0 mL of 4M nitric acid, 3.0 mL of Sample 1 solution and 3.0 mL of 1M potassium thiocyanate were added and the solution was diluted with distilled water to the mark. A blank solution was prepared by diluting of 1.0 mL of 4M nitric acid and 3.0 mL of 1M potassium thiocyanate to 25 mL and the absorption was set to zero with the blank at 480 nm. The absorption of all solutions was measured at this wavelength [17]. The experiment was repeated three times. This experiment was done for Sample 2 similarly. The results are shown in Table 1 and Fig. 1.

Determination of calcium by Atomic Absorption Spectroscopy (AAS) method, plotting a calibration curve

In five 100-mL numbered volumetric flasks, 1.0, 2.0, 3.0, 4.0 and 5.0 mL of 100 ppm calcium standard solution was transferred, respectively. In the 6th flask (is named unknown 1), 1.0 mL of Sample 1 solution and in the 7th flask (is named unknown 2), 10.0 mL of Sample 2 solution was added. All solutions were diluted to the mark with distilled water. Then, the wavelength was set to 422.7 nm and the absorption set to zero with distilled water. Finally, the absorption of all of the solutions was measured three times [17]. Table 2 and Fig. 2 show the results.

Determination of magnesium by Atomic Absorption Spectroscopy (AAS) method, plotting a calibration curve

Five standard solutions were prepared by diluting of 2.0, 3.0, 4.0, 6.0 and 8.0 mL of 10 ppm magnesium standard solution to 100 mL, respectively. In 100- mL volumetric flask number 6 (is named unknown 1), 1.0 mL of Sample 1 and in flask number 7 (is named unknown 2), 10.0 mL of Sample 2 solutions were diluted to the mark with distilled water. The absorption was measured at 285.2 nm for each solution [17]. The results are presented in Table 3 and Fig. 3.

Determination of potassium by Flame atomic Emission Spectroscopy (FES) method, plotting a calibration curve

Three standard solutions, 15, 30, and 45 ppm of potassium were prepared. The sample (unknown) solutions were prepared by diluting of 1.0 mL of Sample 1 and 5.0 ml of sample 2 solutions respectively to 100 mL. Potassium filter was placed in the instrument.

(1) Part per million which is equal to mg per liter for very dilute solutions

Table 1: Iron Standard Addition Curve Data. Note that absorbance is a dimensionless quantity.

Solution number	1	2	3	4	5
Volume of 20 ppm Fe^{3+} solution, mL	0.0	0.5	1.0	1.5	2.0
Standard Fe^{3+} concentration, ppm	0.0	0.4	0.8	1.2	1.6
Mean absorbance (Sample 1)	0.294	0.335	0.375	0.421	0.461
Mean absorbance (Sample 2)	0.186	0.231	0.271	0.320	0.379

Table 2: Calcium Calibration Curve Data. Note that absorbance is a dimensionless quantity.

Solution number	1	2	3	4	5	6	7
Standard Ca^{2+} concentration, ppm	0	1	2	3	4	Unknown(1)	Unknown(2)
Mean absorbance	0.000	0.2015	0.3629	0.5008	0.6401	0.2142	0.3015

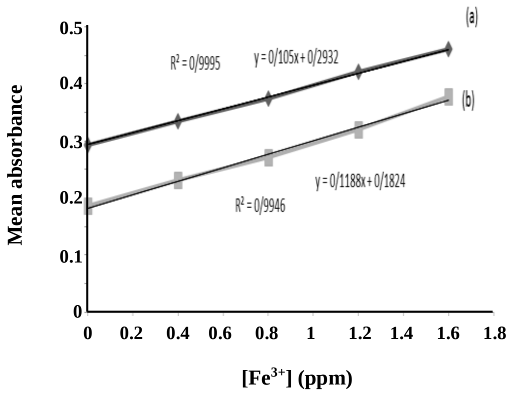

Fig. 1: Iron Standard Addition Curve for (a) Sample 1 and (b) Sample 2.

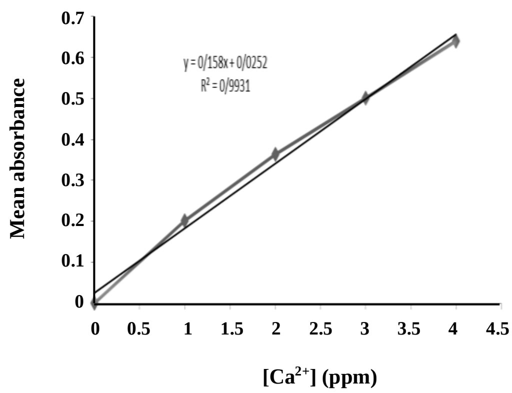

Fig. 2: Calcium Calibration Curve.

The relative emission intensity of the apparatus adjusted to 0 with distilled water and to 100 with 45 ppm solution. The relative emission intensity of the standard and samples solutions were measured [18]. The results are shown in Table 4 and Fig. 4.

RESULTS AND DISCUSSION

In this section, results obtained from different methods are discussed. The experimental results showed that: (1) the appearance of the Dooshab is different from what is produced from skipping the mud-boiled syrup process (resulting in the production of grape sauce). (2) The Dooshab is red, clear and homogeneous while the grape sauce is brown, turbid and inhomogeneous. (3) The taste of the Dooshab is sweet but the grape sauce tastes tart. (4) The acidity of the Dooshab is lower than the grape sauce. (5) The amount of iron, calcium, magnesium, and potassium in the Dooshab is significantly higher than the grape sauce.

Calculating the Amount of Iron in the Samples

The measured iron data are listed in Table 1. Plotting the average absorbance value versus the standard iron solution concentration ($[Fe^{3+}]$) for Sample 1 yields a line with an equation of y=0.105x+0.2932, R^2=0.9995, and intercept of 0.3 (Fig. 1-a). Extrapolation (y=0) gives 2.79 ppm for the concentration of the iron in 25 mL of the tested solution which is equivalent to 3 ml of the Sample 1 solution. Therefore the iron concentration ($[Fe^{3+}]$) in the Sample 1 solution is:

$$\frac{2.79\text{ppm Fe}\times 25\text{mL}}{3\text{mL}} = 23.25\text{ppm Fe}$$

The iron weight in 25 mL of the Dooshab sample is obtained according to:

Table 3: Magnesium calibration curve data. Note that absorbance is a dimensionless quantity.

Solution number	1	2	3	4	5	6	7
Standard Mg^{2+} concentration, ppm	0.0	0.2	0.4	0.6	0.8	Unknown(1)	Unknown(2)
Mean absorbance	0.0000	0.1450	0.2781	0.4070	0.5244	0.4557	0.4347

Table 4: Potassium calibration curve data. Note that emission intensity is a dimensionless quantity.

Solution number	1	2	3	4	5	6
Standard K^+ concentration, ppm	0	15	30	45	Unknown(1)	Unknown(2)
Relative emission intensity	0	38	74	100	59	76

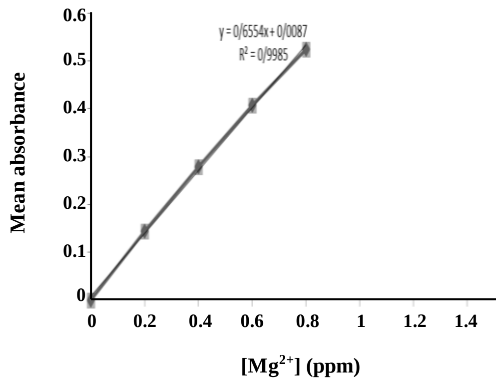

Fig. 3: Magnesium Calibration Curve.

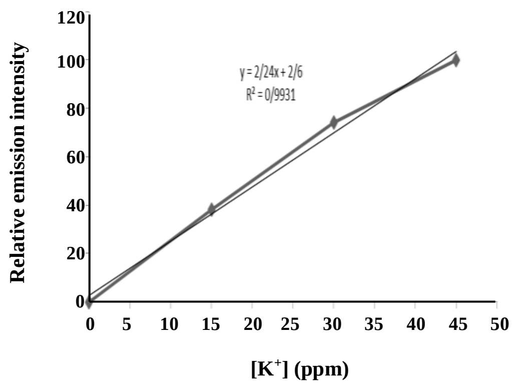

Fig. 4: Potassium Calibration Curve

$$\frac{23.25\text{mg Fe}}{1\text{L Sample1}}\times\frac{0.1\text{L Sample1}}{25\text{mL Dooshab}}=\frac{2.32\text{mg Fe}}{25\text{mL Dooshab}}$$

Similarly for Sample 2, y=0.1188x+0.1824, R^2=0.9946 and the intercept is 0.182 (Fig. 1-b). Extrapolation gives 1.535 ppm for iron concentration in 25 ml of the tested solution, thus the iron concentration in sample 2 solution is:

$$\frac{1.535\text{ppm Fe}\times 25\text{mL}}{3\text{mL}}=12.8\text{ppm Fe}$$

And the iron weight is:

$$\frac{12.8\text{mg Fe}}{1\text{L Sample 2}}\times\frac{0.1\text{L Sample 2}}{25\text{mL grape sauce}}=\frac{1.28\text{mg Fe}}{25\text{mL grape sauce}}$$

Calculating the amount of calcium in the samples

Table 2 shows the results of calcium determination by the Atomic Absorption Spectroscopy (AAS) technique. The curve of mean absorbance versus the standard calcium concentration ($[Ca^{2+}]$) is a straight line with an equation of y=0.158x+0.0252 and R^2=0.9931. Putting the absorbance value of samples gives 1.20 and 1.75 ppm of calcium concentration for 100 mL of unknown solutions that contain 1.0 mL and 10.0 mL of sample solutions, respectively.

The calcium concentration in Sample 1 is:

$$\frac{1.20\text{ppm}\times 100\text{mL}}{1\text{mL}}=120\text{ppm}$$

and the calcium weight in 25 ml of the Dooshab sample is:

$$\frac{120\text{mg Ca}}{1\text{L Sample 1}}\times\frac{0.1\text{L Sample 1}}{25\text{mL Dooshab}}=\frac{12\text{mg Ca}}{25\text{mL Dooshab}}$$

the calcium concentration in Sample 2 is:

$$\frac{1.75\text{ppm}\times 100\text{mL}}{10\text{mL}}=17.5\text{ppm}$$

and the calcium weight in 25 mL of the grape sauce sample is:

$$\frac{17.5\text{mg Ca}}{1\text{L Sample 2}} \times \frac{0.1\text{L Sample 2}}{25\text{mL grape sauce}} = \frac{1.75\text{mgCa}}{25\text{mL grape sauce}}$$

Calculating the Amount of Magnesium in the Samples

The results of magnesium determination by AAS are shown in Table 3 and Fig. 3. The calibration curve is a line with an equation of y=0.6554x+0.0087 and R^2=0.9985. Putting the absorbance value of unknown absorptions in this equation gives 0.682 and 0.650 ppm for magnesium concentration in those solutions. The amount of magnesium in 100 mL of unknown 1 solution which is equivalent to 1 mL of Sample 1 solution is:

$$\frac{0.682\text{ppm Mg} \times 100\text{mL}}{1\text{mL}} = 68.2\text{ppm Mg}$$

and the weight of magnesium in 25 mL of the Dooshab is:

$$\frac{68.2\text{mg Mg}}{1\text{L Sample1}} \times \frac{0.1\text{L Sample1}}{25\text{mL Dooshab}} = \frac{6.82\text{mg Mg}}{25\text{mL Dooshab}}$$

Similarly, the amount of magnesium in 100 mL of unknown 2 solution which is equivalent to 10 mL of sample 2 solution is:

$$\frac{0.650\text{ppm Mg} \times 100\text{mL}}{10\text{mL}} = 6.5\text{ppm Mg}$$

and the weight of magnesium in 25 mL of grape sauce is:

$$\frac{6.5\text{mg Mg}}{1\text{L Sample 2}} \times \frac{0.1000\text{L Sample 2}}{25\text{mL grape sauce}} = \frac{0.65\text{mg Mg}}{25\text{mL grape sauce}}$$

Calculating the amount of potassium in the samples

The data of potassium measurement are listed in Table 4. Plotting means relative emission intensity versus standard potassium solution concentration ([K^+]) gives a linear curve with an equation of y=2.24x+2.6 and R^2=0.9931. According to relative emission intensity, [K^+] in solutions is obtained: 25.0 ppm in unknown(1) and 32.5 ppm in unknown(2). The concentration and weight of potassium in samples considering dilution factors are:

$$\frac{25\text{ppm K} \times 100\text{mL}}{1\text{mL}} = 2500\text{ppm K},$$

$$\frac{2500\text{mg K}}{1\text{L Sample1}} \times \frac{0.1000\text{L Sample1}}{25\text{mL Dooshab}} = \frac{250\text{mg K}}{25\text{mL Dooshab}}$$

$$\frac{32.5\text{ppm K} \times 100\text{mL}}{5\text{mL}} = 650\text{ppm K}$$

$$\frac{650\text{mg K}}{1\text{L Sample 2}} \times \frac{0.1000\text{L Sample 2}}{25\text{mL grape sauce}} = \frac{65\text{mg K}}{25\text{mL grape sauce}}$$

CONCLUSIONS

Methods used in this study, i.e. absorption spectrophotometry, atomic absorption spectroscopy, and flame atomic emission spectroscopy, are rapid, sensitive and precise techniques for determining elements in real samples. We list the main lessons learnt from these experiments:

- The mud boil-up process causes differences in the appearance and taste of the Dooshab and grape sauce. The clarity of the syrup after the mud boil-up process may be attributed to the fact that the colloidal substances are adsorbed by the ions in the soil (during juice boiling with soil) in particular iron, which results in the precipitation of those particles as sludge.
- The acidity of grape juice is decreased by alkaline salts in the white soil resulting in its sweet flavor.
- It seems that contact of grape juice with soil during mud boil-up process and cooling down step result in ion transferring from the soil to the syrup.

Nutritionists advise people with iron deficiency anemia and arthritis to use grape syrup and whom with hyperkalemia not to use it an argument that would be strengthened with our results. This research proved that elements have an important role in human health, such as iron, calcium, potassium and magnesium transfer from the soil into the syrup.

In conclusion, our study demonstrates that Dooshab is a good source of iron, calcium, and magnesium. It is also a rich source of potassium.

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