

Effect of Using Grape Molasses as Anti Anemic

Makpoul .K .R

Food Science and Technology - Agricultural Industrializationunit, Department of Plant Production - Desert Research Center – Cairo – Egypt

Abstract: *The grape molasses was evaluated for its anti-anemic activity in rats. The effect of grape molasses dose on hemoglobin concentration, red blood cell count and packed cell volume was investigated. The doses 3 ml, 6 ml and 9 ml per day demonstrated anti-anemic property by significantly ($p < 0.05$) for 20 days increasing the levels of red blood cell count, hemoglobin level and packed cell volume of treated animals. The effects of grape molasses on iron in rats were investigated, Phytochemical screening of the grape molasses revealed the presence amount of iron, and thus lend credence to its use in the management of anemia.*

Keywords: grape, juice, molasses, anemia, hemoglobin concentration, red blood cell count, packed cell volume

1. Introduction

Micronutrient deficiencies are still a major public health a problem in many developing countries with infants and pregnant women usually at risk. Infants warrant extra concern because they require extra micronutrients to maintain optimal growth and development (Oladijiet al, 2007). Iron one of micronutrient is important for cognitive performance, work capacity, immunity to infections. Iron deficiency is the most common single-nutrient deficiency disease in the world (Buzina et al, 1998). affecting about 15% of the world population (Eftekhari et al 2003). 35% of women and 43% of young children (Sharmanov1998).

Iron deficiency usually develops gradually and begins with a negative iron balance or when iron intake does not meet the body's daily iron needs. This negative balance initially depletes the stored form of iron while the blood hemoglobin level, a marker of iron status, remains normal. Iron deficiency anemia is an advanced stage of iron depletion. It occurs when storage sites of iron are deficient and blood levels of iron cannot meet daily needs. Blood hemoglobin levels are below normal with iron deficiency anemia. Iron deficiency anemia can be associated with low dietary intake of iron, inadequate iron absorption or excessive blood loss (Batra & Seth, 2002).

Grapes *Vitisvinifera* and grape products have an economic value where they are mostly consumed as table grapes, grape juice or raisins. Grape is produced widely in Egypt, and it is nutrition as well as a source of income for people. On account of its health benefits and short shelf life, grapes are required to be processed into a form enabling long storage time without loss of nutritional value (Eksi & Artık1984, Tangolar&Ergenoglu1996). Balady black grapes from Sinai-Egypt may vary appreciably in the composition of certain specific constituents, which define decisively the overall quality of molasses. Grape juice is concentrated (up to 70–80% of soluble dry matter) to obtain molasses (Bozkurt et al 1999). It is a very important food in human nutrition because molasses have a high sugar content of monosaccharides such as glucose and fructose and organic acids (Dietary Reference Intakes for Micronutrients 2006). Also, it has high amounts of mineral especially an excellent source of iron (Akbulut&Özcan2008, Tosun & Üstün, 2003). Although various components have been thoroughly studied with respect to their impact on the quality of grape molasses, only

relatively recently have these components gained increasing interest as nutritional anti-anemic. One of the major parameters that are of both technological and nutritional significance is the iron composition.

2. Materials and Methods

2.1 Grape Molasses Production

These grapes are locally named as black grapes. 100 kilograms fresh grapes were used. Grapes were washed for extracting of rotten, moldy and damaged parts of them before extract juice. Grape pulp and juice easily were separated from each other during extraction. 50 kilograms of grape juice were obtained from 100 kilograms grapes. 50 kgs of juice extracted evenly divided into two outdoor boiling containers, each container contain 25 kg juice, was made ready for the production of grape molasses. Grape must in the heated form in boiling containers was transferred to the large plastic containers and then was subjected to the process of grounding. Grape juice was divided into two phases, which are with sediment and without sediment. The clear phase, which was at the top of the plastic containers, was taken to the boiling containers again. During the boiling process, dull and dirty foams occurred. Dirty foams were taken with a wood spoon from the surface of boiling containers. The boiling processes of grape molasses took 4 hours for every container. The boiling containers were taken from the top of the stoves to cool them down. 10 kilograms of grape molasses were obtained from 50 kilograms grape juice (Mehmet et al, 2015).

2.2 Chemical Analyses

The grape molasses and grape juice were analyzed at room temperature. Water soluble dry matter, pH, hydroxymethyl furfural (HMF), protein, ash, invert and total sugars of the samples were determined. Water soluble dry matter, crude protein, fat, fiber, and ash were determined according to standard AACC (1990) methods; pH and water soluble dry matter were determined according to (Cemerog1992). pH was measured with a pH meter (WTW-315 model, GmbH, Weilheim, Germany); soluble dry matter was determined with an ATAGO model refractometer. Total sugar and invert sugar were quantitated by the Lane-Eynon method (Cemerog1992).

Hydroxymethylfurfural (HMF) was determined quantitatively following the procedure described by the IFJJP (1964). based on the colorimetric reaction between barbituric acid, p-toluidin and HMF, forming a red-colored complex. The intensity red color was measured at 550 nm by using Shimadzu UV-Visible 160 a model spectrophotometer (Shimadzu Corporation, Tokyo, Japan).

2.3 Determination of mineral contents

Mineral matters of samples were found by using the ICP-AES (Inductively Coupled Plasma- Atomic Emission Spectrometer) device and the values are given to be ppm (mg/kg). The results were found over from Ca, Cu, Fe, Mg, Mn, Na, P and Zn mineral substances.

About 0.5 g of each grape molasses and grape juice sample was put into a burning cup and 15 ml pure HNO₃ were added. The sample was incinerated in a MARS 5 Microwave Oven at 200 °C and dissolved ash diluted to a certain volume with water. Distilled deionized water and ultrahigh-purity commercial acids were used to prepare all reagents, standards, and samples. After digestion treatment, samples were filtrated through whatman No 42. The filtrates were collected in 50 ml flasks and analyzed by ICP-AES. The mineral contents of the samples were quantified against standard solutions of known concentrations which were analyzed concurrently (Skujins1998).

2.4 Animal grouping and feed protocol

Fifty (50) Healthy male rats (250 to 300 g) were selected for the experiment, The rats were divided into five groups each group consists of ten rats. The animals were individually housed in metabolic cages of dimensions 33 cm×20.5 cm×19 cm under standard conditions (12-h light:12-h dark, 28 °C±3 °C and 40–55% humidity). They were allowed free access to normal rat chow and distilled. The acclimatization was done for seven days before the start of the experiment and Fed groups for 20 days explained below:

- Group (1) was fed by basic meals only without induction of anemia and was designated as normal control (NC).
- Group (2) was fed by basic meals with induction of anemia and was designated as anemic control (AC).
- Group (3) received 1 ml of grape molasses three time (3 ml per day) before fed by basic meals with induction of anemia, designated as grape molasses 1 (GM1).
- Group (4) received 2 ml of grape molasses three time (6 ml per day) before fed by basic meals with induction of anemia, designated as grape molasses 2 (GM2).
- Group (5) received 3 ml of grape molasses three time (9 ml per day) before fed by basic meals with induction of anemia, designated as grape molasses 3 (GM3).

2.5 Induction of Anemia and Hematological test

The baseline hematological parameters were assayed on the rats before the induction of anemia. Anemia was induced according to the method described by Stone (Stone 1954). About 1 ml of blood was removed from each rat daily for about 5 days until the rats became anemic. Anemia was induced in all the groups except Group 1 (Normal Control) and their hematological parameters determined after the induction of anemia to ascertain the level of anemia in the rats. The following hematological parameters;

- Packed Cell Volume (PCV)
- Hemoglobin Concentration (Hb)
- Red Blood Cell Count (RBC)
- hematological parameters differential every five days of treatment with the extract using the methods described by (Ochei & Kolhatkar 2008).

2.6 Statistic Analysis

All data were expressed as mean ± SEM value. The significant differences among various groups were compared by ANOVA and followed by Duncan's test. The statistical difference was regarded at p<0.05.

3. Results and Discussion

3.1 Chemical Analyses

The results of physical and chemical analyses on black grape molasses that was produced are given as follows (Fig 1). Accordingly, the results showed differences in physical and chemical properties of grape juice and grape molasses. While pH value varied between 4.87 and 5.29, Protein rate was changed between 0.85 and 0.80 percent at the final product. Total sugar was varied between 16.2 and 72.4, Invert sugar increased from 13.9 to 59.7 during the process stages of grape molasses production. Water-soluble dry matter rate was raised from the juice stage (15.7) towards to the final product stage (74.3) due to the increase in the concentration by the effect of heat treatment. Generally, molasses is produced from sugar-rich fruits by the concentration of juices up to 70–80% soluble dry matter content (Alpaslan. & Hayta 2002). Different researchers found soluble dry matter values between 52 and 81% in 76 grape molasses samples (Aktan 1940, Batu 1991, Yaziciog & Gökçen 1976). Ash, fat and fiber content changed between 0.9 to 1.7%, 0.8 to 1.4% and 4.3 to 8.6 respectively. HMF content of grape molasses (34.1 mg/L) was the higher than grape juice. HMF is an indicator of quality deterioration, which occurs as a result of excessive heating in food (Cemerog1992). Maillard reactions result in undesirable color, odor and flavor changes and are followed by the formation of intermediates such as 5- hydroxymethyl furfural (HMF), particularly under acidic conditions and finally brown pigment formation (Bozkurt & Eren1999).

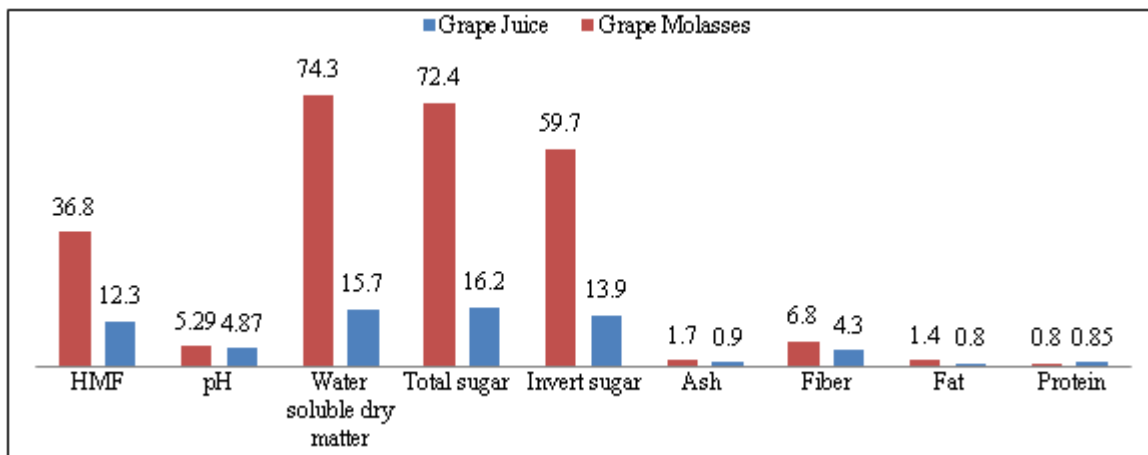


Figure 1: Some physicochemical properties of red grape molasses compared with grape juice

3.2 Mineral Contents

Mineral matter values (mg/kg) of black grape molasses were given in Fig 2. When the stages of processing black grape molasses were identified at the juice and final product stages. The presence of Calcium mineral identified at the stages of juice 47 mg/kg and 970 mg/kg In the last stage of grape molasses. Magnesium mineral was found 32 mg/kg in juice and 198 mg/kg at the stage of the final product of grape molasses. The amount of Zinc, Sodium and copper were found less than the amount of the other elements in final black grape molasses 1.49, 1.2 and 0.67 mg/kg respectively. Also, the amount of manganese mineral was found 0.96 mg/kg in juice and 2031 mg/kg at the stage of final grape molasses. The content of Phosphor mineral was found 114 mg/kg in juice and 298 mg/kg in grape molasses. The high

iron content makes it a recommended treat for anemia (Arslan & Esin 2005). Iron was found 137 mg/kg in juice stage and the amount of iron mineral was increased due to the increase in the concentration to 342 mg/kg in grape molasses. Potassium mineral was found 93 mg/kg in juice stage. The amount of Potassium mineral was increased due to the increase in the concentration by the effect of heat to 1980 mg/kg in grape molasses. In black grape molasses, the higher values in terms of mineral content were potassium, calcium and iron mineral. Grape molasses is a rich source of iron content 10.26 mg/30ml essential to human body as compared to the related RDAs 10mg/d (Recommended dietary allowances for children four to eight years) Dietary Reference Intakes for Micronutrients (2006).

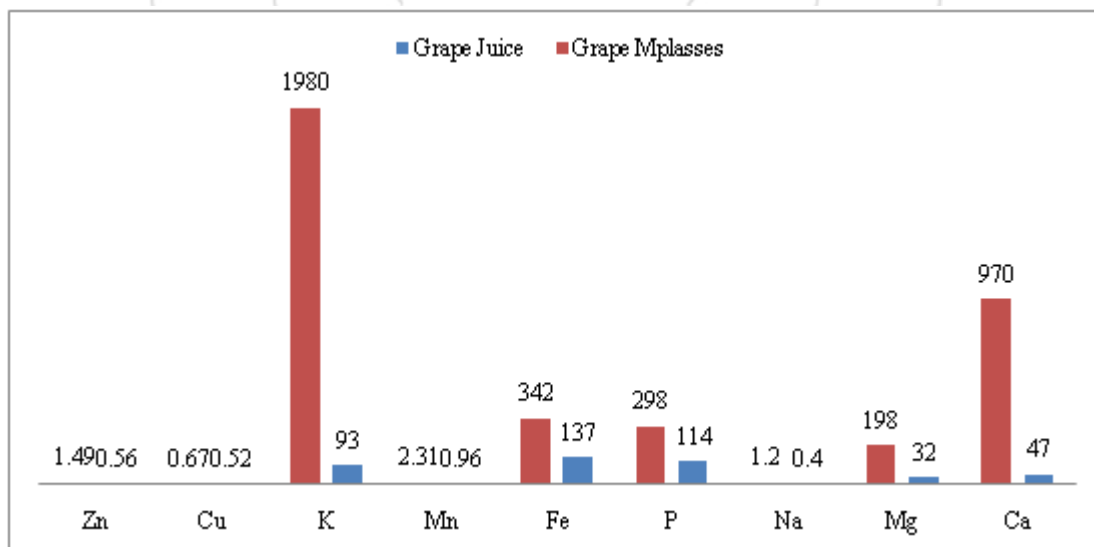


Figure 2: Mineral matter content of red grape molasses compared with grape juice

3.3 Anemia and Hematological

The results of the effect of grape molasses on anemic of rats are presented in fig 3,4 and 5. The result showed that there was improvement in hematological indices that are indicative of anemia in tested groups ($p < 0.05$) when compared to the anemic control. It can be deduced from result that the administered grape molasses was able to alleviate induced anemia in experimental animals. The world

health organization (WHO, 2008), defines anemia as hemoglobin levels less than 13 g/dL. Groups GM1, GM2 and GM3 animals treated with 1ml, 2ml and 3ml of the grape molasses respectively had a significantly increased hemoglobin levels ($p < 0.05$) when compared to the anemic control group (AC) and non-treated group (NC). Also, the increased RBC and PCV in the treated groups when compared to both anemic and normal control is an indication that the grape molasses improved anti-anemic indices in the

experimental animals. This effect could be due to the presence high amount of iron in grape molasses. Usual therapy depends upon the nature and type of anemia and revolves around the use of hematopoietic growth factors,

minerals or vitamins supplementation as Iron or Iron salt (Hillman 2001).

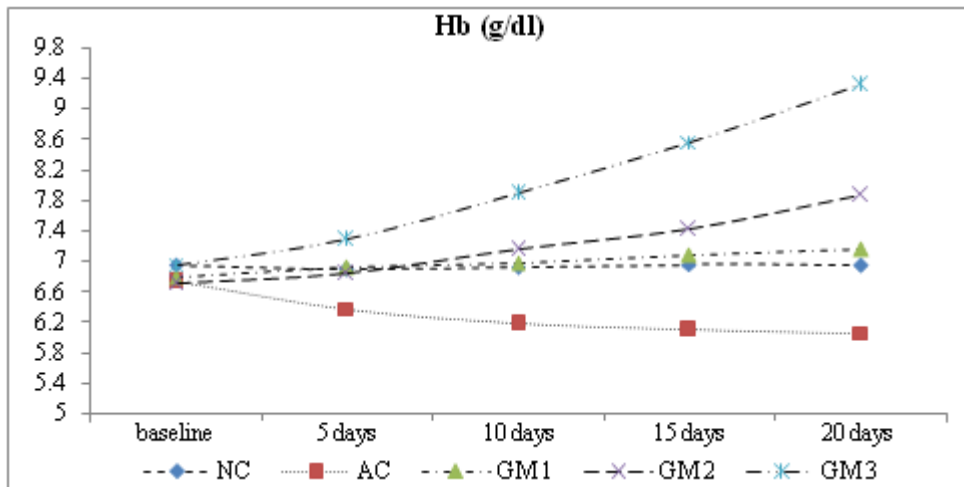


Figure 3: Effect of grape molasses on hemoglobin concentration (Hb g/dl) in anemia infected rats (NC) normal control, (AC) anemic control, (GM1) received 3 ml/day of grape molasses, (GM2) received 6 ml/day of grape molasses, (GM3) received 9 ml/day of grape molasses. Data were presented as mean \pm SEM P-value < 0.05

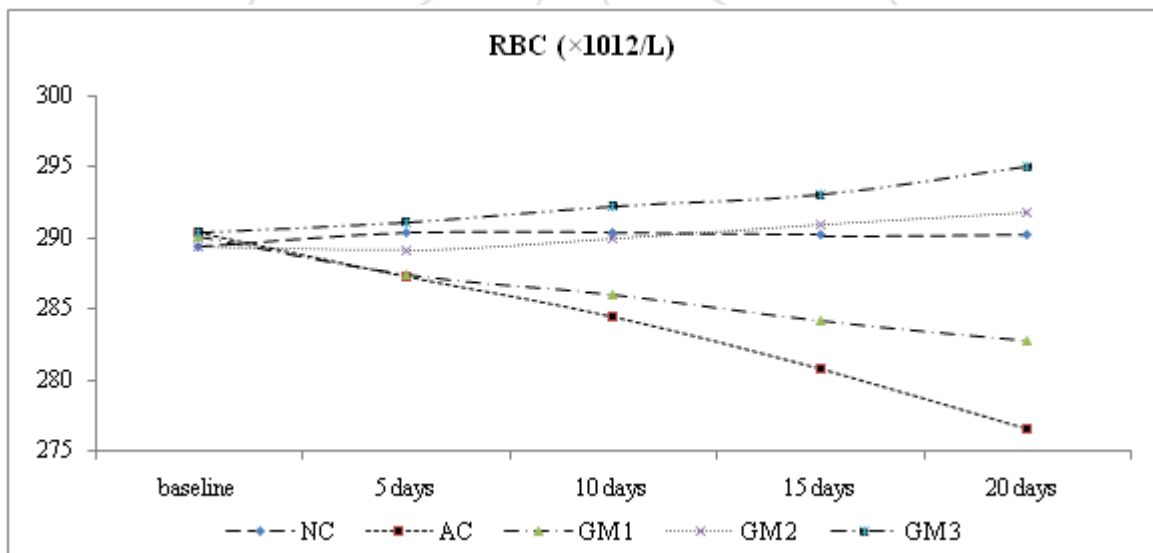


Figure 4: Effect of grape molasses on Red Blood Cell count (RBCx1012/L) in anemia infected rats. (NC) normal control, (AC) anemic control, (GM1) received 3 ml/day of grape molasses, (GM2) received 6 ml/day of grape molasses, (GM3) received 9 ml/day of grape molasses. Data were presented as mean \pm SEM P-value < 0.05

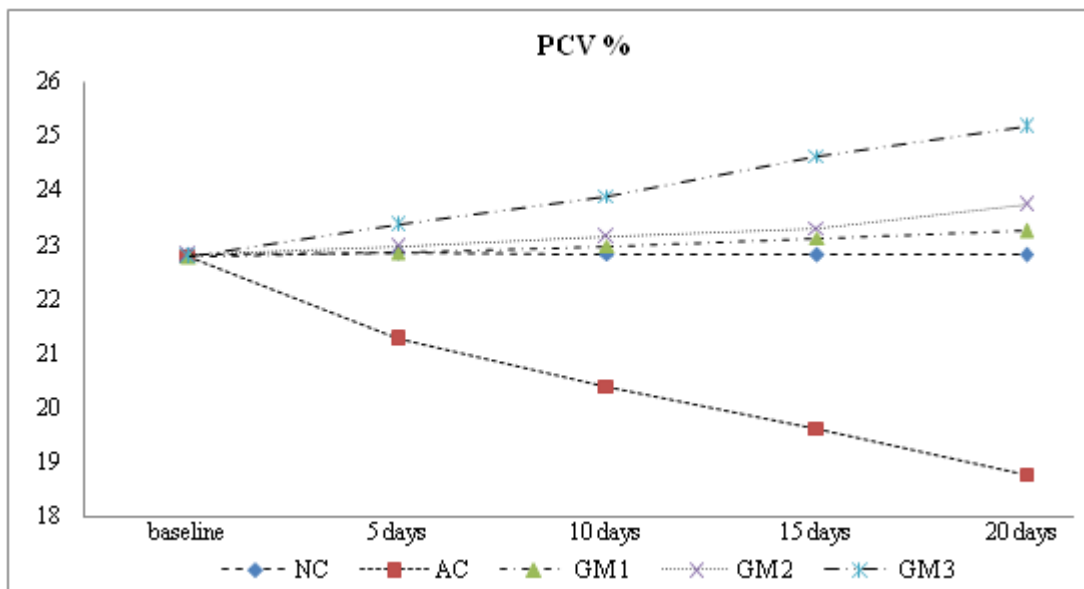


Figure 5: Effect of grape molasses on Packed Cell Volume (PCV%) in anemia infected rats.

(NC) normal control, (AC) anemic control, (GM1) received 3 ml/day of grape molasses, (GM2) received 6 ml/day of grape molasses, (GM3) received 9 ml/day of grape molasses. Data were presented as mean \pm SEM P-value < 0.05

4. Discussion

The most reliable indication of iron deficiency anemia is hemoglobin. This is because it is the iron-containing protein found in red blood cells that allows the red blood cells to function as the oxygen transport system to the tissues of the body. Next to hemoglobin in this regard is the packed cell volume (PCV) which is a measure of the portion of the blood volume made up by red blood cells. From Fig 3, 4 and 5 the significant decrease in hemoglobin, PCV and RBC levels of iron deficient rats may be attributed to induction of iron deficiency anemia when rats were placed on iron-deficient diet as is the case in this study. However, grape molasses resulted in significant increases in hemoglobin, PCV and RBC levels in all the dose groups. Such increase in the blood parameters further lend credence to the acclaimed use of the grape molasses as anti-anemic agent since the Hb, PCV and RBC levels of iron-sufficient rats were also increased by treatment with all the three doses of the grape molasses. Iron serves as the core of hemoglobin molecule, which is the oxygen-carrying component of red blood cells. The ability of red blood cells to carry oxygen is attributed to the presence of iron in the hemoglobin molecule. A lack or loss of iron by any means implies reduced production of hemoglobin and subsequent reduction in the volume of red blood cells. Hence, it may be concluded that dietary iron deficiency led to a decrease in the iron-containing protein, hemoglobin, and by extension reduction in red blood cells and PCV levels. Based on the present study, it is most likely that the hemoglobin restoring and anti-anemic effects of grape molasses may be made possible by the presence of iron.

5. Conclusion

This study has lent credence to the use of the grape molasses in the treatment and management of anemia. This may have been possible due to the presence of some blood boosting components in the grape molasses. The grape molasses has been used as iron supplement, where many of its nutritional

properties have been harnessed. Since anemia has been implicated in the etiology of many diseases, the consumption of grape molasses for its nutritional and medicinal properties is therefore strongly recommended.

References

- [1] A.T. Oladiji *, T.O. Jacob, M.T. Yakubu, (2007). Anti-anaemic potentials of aqueous extract of Sorghum bicolor (L.) moench stem bark in rats. *Journal of Ethnopharmacology* 111, 651–656
- [2] AACC. (1990). *Approved Methods of the American Association of Cereal Chemists*, 8th Ed., AACC, St. Paul, MN.
- [3] Akbulut, M. And Özcan, M.M. (2008). Comparison of mineral contents of mulberry (*Morus spp.*) fruits and their pekmez (boiled mulberry juice) samples. *Int. J. Food Sci. Nutr.* doi:10.1080/09637480701695609 (in press).
- [4] Aktan, R. (1940). Üzümpekmezleriüzerineteknikaras, tirmalar. (Technical researches on grape pekmezes). *ZiraatDergisi*1(12), 12–28 (in Turkish).
- [5] Alpaslan, M. And Hayta, M. (2002). Rheological and sensory properties of pekmez (grape molasses)/tahin (sesame paste) blends. *J. Food Eng.* 54, 89–93.
- [6] Arslan, E., Yener, M.E. and Esin, A. (2005), “Rheological characterization of tahin/pekmez (sesame paste/concentrated grape juice) blends”, *J. Food Eng.*, Vol. 69, pp. 167-172.
- [7] Batra, J., Seth, P.K., (2002). Effect of iron deficiency on developing rat brain. *Indian Journal of Clinical Biochemistry* 17, 108–114.
- [8] BATU, A. (1991). A research on preliminary processes applied to raisin juice that will be produced into pekmez. *Cum. Univ. J. Tokat Agric. Fac.* 7(1), 191–202.
- [9] Bozkurt, H., Göğüş, F., & Eren, S. (1999). Nonenzymic browning reactions in boiled grape juice and IST models during storage. *Food Chemistry*, 64, 89–93.

- [10] **Buzina-Suboticanea, K., Buzina, R., Staulijenic, A., Tadinac-Babic, M., Juhovic-Markus, V., (1998).** Effects of iron supplementation on iron nutrition status and cognitive functions in children. *Food and Nutrition Bulletin* 19, 298–306.
- [11] **Cemerog̃ Lu, B. (1992).** MeyveveSebzels,lemeEndüstrisindeTemelAnalizMetotlari. (Basic Analysis Methods in Fruit and Vegetable Processing Industry), Biltav Press, Ankara, Turkey (in Turkish).
- [12] **Dietary Reference Intakes for Micronutrients (2006),** available at: <http://fnic.nal.usda.gov/dietaryguidance/dietary-reference-intakes>.
- [13] **Dogan, M. (2007).** Rheological and physicochemical characteristics of ice cream mix with molasses in a model system. *Milchwissenschaft (Milk Science International)*, 62(2), 195–198.
- [14] **Eftekhari, M.H., Connor, J.R., Beard, J.L., Malecki, E.A., Epstein, D.K., Allen, R.P., (2003).** Abnormalities in cerebrospinal fluid concentrations of ferritin and transferrin in RLS. *Neurology* 54, 1698–1700.
- [15] **Eksi, A., &Artık, N. (1984).** Rheology of food materials. *Current Opinion in Colloid & Interface Science*, 16, 36–40.
- [16] **Hillman RS (2001).** Hematopoietic Agents: Growth factors, Minerals and vitamins. In Hardman JG, Limbird LE, ed. *The pharmacological basis of therapeutics*. 10th Edition New York. McGraw. Hill pp. 1487-1517.
- [17] **IFJJP. (1964).** International Federation of Fruit Juice Producers Methods, Analysis; Fruit-Union Suisse Assoc., Zug, Switzerland.
- [18] **Mehmet Musa Özcan&ŞerifeAlpar& Fahad AL Juhaimi, (2015).** The effect of boiling on qualitative properties of grape juice produced by the traditional method. *J Food SciTechnol* 52(9):5546–5556
- [19] **Ochei J, Kolhatkar A (2008).** *Medical Laboratory Sciences: Theory and Practice*. Tata McGraw Hill, New York, pp 663-665.
- [20] **Sharmanov, A., (1998).** Anaemia in central Asia: demographic and health survey experience. *Food and Nutrition Bulletin* 19, 307–317.
- [21] **Skujins S (1998)** Handbook for ICP-AES (Vartian-Vista). A Short Guide to Vista Series ICP-AES Operation. Variant Int. AG, Zug version 1.0 Switzerland.
- [22] **Stone SH (1954).** Method of obtaining venous blood from the orbital sinus of rats or mouse. *Science* 119:100-102.
- [23] **Tangolar, S., &Ergenoglu, F. (1996).** ÜzümÇeşitleriKatalogu. Adana: ÇukurovaÜniversitesiZiraatFakültesi Offset Atölyesi.
- [24] **Tosun, I. And Üstün, N.S. (2003).** Nonenzymatic browning during storage of white hard grape pekmez (Zilepekmezi). *Food Chem.* 80, 441– 443.
- [25] **Yaziciog̃ Lu, T. And Gökçen, J. (1976).** Kuru Üzümnden Difüzyon Yoluile Pekmez Elde EdilmesiI'çinGelis, tirilen Bir Yöntem. (An Improved Method for Obtaining Pekmez from Grape with Diffusion Method), Tubitak Press, Gebze, Turkey.