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Effect of different clarifying agents on the amount of essential elements of grape juice concentrate

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ABSTRACT

Grape juice concentrate is a traditional product of grape-harvesting areas of Iran which is generally produced from the year-end harvest of poor quality. Average of iron, copper and calcium in the grapes is 30, 6.5 and 180 mg/kg respectively. These elements are essential and useful in human nutrition. In order to examine the effect of different clarifying materials on the amount of essential elements of grape juice concentrate, a plan was executed using factorial statistical method with completely randomized design. The first factor was type of clarifiers in six levels, comprising grape juice concentrate soil (GJCS), bentonit, silicasol, gelatin-bentonit, gelatin-silicasol, gelatin-bentonit-silicasol; and the second factor was the quantity of clarifiers at three levels with three replicate. According to the results obtained from statistical analysis, maximum quantity of iron and copper obtained from Gelatin-Bentonit-Silicasol treatment, and maximum quantity of zinc and magnesium has been resulted from Gelatin-Bentonit treatment and the maximum quantity of calcium has been obtained from Gelatin-Silicasol treatment; the least quantity of iron and calcium has been obtained from Bentonit treatment, the least quantity of copper and zinc has been obtained from GJCS treatment and the least quantity of magnesium has been achieved by Silicasol treatment.

KEYWORDS

Grape juice concentrate;
Clarification;
Grape juice;
Filter aid compounds;
Essential elements

INTRODUCTION

Grape juice concentrate, with the local name of Dooshab, is a traditional product of grape-harvesting areas of Iran, produced from boiling and condensation of grape juice to the brix scale of over 70-80%, in open containers or in vacuum, and without adding sugar or other additives[6,18]. Grape juice concentrate contains high volumes of natural sugar, minerals, vitamins A, B_1, B_2 and C, organic acids and antioxidants. It, therefore, plays an important role in the nutrition of various age groups, especially children and athletes[1,15,28,29]. Grape juice concentrate is quickly absorbed by the body as a result of its high volume of digestible monosaccharide. It is, therefore, useful for those weakened as a result of a chronic disease or after undergoing a medical operation[1,6,18,26,27]. Grape juice concentrate is a rich source of essential elements to human body, such as copper, zinc and iron. Iron contained in grape juice concentrate may be useful in the treatment of anemia patients[4,28].

Copper, zinc, iron, calcium and magnesium are essential elements in human food. They are also necessary and useful for human health. Excessive intake of the said elements, more than standard level causes poisoning in human body[11]. Moreover, the quantity of the said metals plays a great role in quality of grape juice concentrate in such a way as high concentration of such elements as iron, zinc, manganese and magnesium causes instability, opacity and poor quality of grape juice concentrate[24]. Copper, as a catalyst, plays a significant role in oxidizing organic compounds and processing such products as beer and wine. The main source of copper in products such as beer and grape juice concentrate is the equipment used for production. Although zinc has been identified as one of nutrient and essential elements, it may be poisonous depending on its concentration. One kilogram of an adult's body contains about 33 mg of zinc. The said element, as a main ingredient of a few enzymes, is involved in various physiological processes such as protein synthesis and energy metabolism[19].

Clarification ways of grape juice concentrate are similar to those of clarification of grape juice. In fruit juice industry, clarification is a unified process that comprises the elimination of undesired color, aroma and flavor; turbidity; bitterness and gassy[22]. In the process of clarification, clarifiers are utilized which are combined with charged particles of fruit juice such as protein, pectin and phenolic materials and are consequently separated from the environment. Usual clarifiers in fruit juice industry are bentonit, gelatin and silicasol. Bentonit is a kind of clay of montmorillonite group with the characteristic of shallow absorption surficial absorption, and affects proteins, poly-phenolic materials, metal ions and the rest of the toxics[9]. The soluble protein gelatin is obtained through relative hydrolysis of collagen existing in animal skin, bones and cartilage. In terms of extraction method, gelatin is divided into acid (A) and alkaline (B) variants[25]. Gelatin characteristics include decreasing the quantity of polyphenols and pectin, making complex with natural proteins of fruit juice and brightening the color of fruit juice. Silicasol is another clarifier which helps to brighten the color of the fruit juice through creating negative charge in fruit juice and flocculating with positively charged compounds[9,22].

This study also made use of a certain white soil called grape juice concentrate soil as the clarifier material in the production of grape juice concentrate. In addition to depositing suspending material, the soil neutralizes the acidity of the grape juice[30]. Bodbodak et al. (2009) studied the effect of different clarification treatments on the physicochemical and rheological characteristics of pomegranate juice. Rai et al. (2007) studied the effect of clarifiers on the quality of mosambi orange juice. Gockmen et al. (2001) and OSzmianski and Wojdylo (2007) studied the effect of clarifiers on the quality of apple juice clarification. Ehteshami Moeinabadi et al. (2005) used sodium carbonate to reduce acidity and bentonit as clarifier in producing grape juice concentrate. Demirozu et al. (2002) studied the quantity of such elements as iron, copper, and zinc in grape juice concentrate; Galbani et al. (2010) studied the quantity of essential elements (iron, copper and zinc) in commercial fruit juices in Pakistan and Nascentes et al (2005) studied the quantity of such elements as copper, manganese, zinc and lead in bear; Schiavo et al (2008) studied the quantity of lead, cadmium, and copper in wine and grape juice.

Relying on revision of respective sources, in this research, it has been tried to make use of the clarifier materials, which have not been used in producing grape juice concentrate yet and to study the effect of the said materials on quantity of such elements as iron, copper, zinc, calcium and magnesium, which play a prominent role in quality of grape juice concentrate because absorption of the said elements by human body is mainly done through food (foods, beverages and water). Thus, due to importance of nutritional value of the said elements and effect of the same on quality of products, measurement of the quantity of the said elements in foodstuff products must be studied accordingly[11].

MATERIALS AND METHODS

Materials

Grape (Razeghi variety) was harvested from the gardens of Nazloochay district in Urmia. Material used for clarification including bentonit (SIHA, Paranit Na-Cabentonit), gelatin (mesh 35, type A, bloom 80, DGF Stoess), commercial silicasol 15% (Baykisol 15%) and calcium carbonate (Charleaux brand, EU0) was provided by Saroone Co. Urmia. Also, GJCS was obtained from the grape juice concentrate producers' bazaar in Urmia.

In order to measure respective metals in grape juice concentrate samples, concentrated Nitric Acid of 65% and Standard Stock Solution of 1000 mg/liter with respect to such metals as iron, copper, zinc, calcium and magnesium of high purity, mark: Merck, have been used. Moreover, in order to specify quantities of metals in the said sample, flame atomic absorption spectrophotometry device, Mark: Perkin Elmer, Model an Analyst 400, has been used. Moreover, in order to supply energy, multi-element lamps (Lumina Lamp) have been used accordingly.

Methods

Production of grape juice concentrate

Fifty four samples of grape juice concentrate (including six treatments in three levels and three repetitions) produced around early October 2011 in the research center of ministry of agriculture in Urmia (TABLE 1). For each sample, about 5 liters of grape juice squeezed from 10 kg of grape by a juicer (Toshiba, Japan), and the pH, acidity and brix of the juices were measured.

TABLE 1 : Clarifying agents used in grape juice concentration

Treatments[a]	Clarifying agent	Concentration
T_{1-A}	Soil	3(g/100ml)
T_{1-B}	Soil	4(g/100ml)
T_{1-C}	Soil	5(g/100ml)
T_{2-A}	Bentonit	4(g/lit)
T_{2-B}	Bentonit	5(g/lit)
T_{2-C}	Bentonit	6(g/lit)
T_{-A}	Silicasol	5(ml/lit)
T_{3-B}	Silicasol	6(ml/lit)
T_{3-C}	Silicasol	7(ml/lit)
T_{4-A}	Gelatin + Bentonit	2(g/lit) + 4(g/lit)
T_{4-B}	Gelatin + Bentonit	2(g/lit) + 5(g/lit)
T_{4-C}	Gelatin + Bentonit	2(g/lit) + 6(g/lit)
T_{5-A}	Gelatin + Silicasol	2(g/lit) + 5(ml/lit)
T_{5-B}	Gelatin + Silicasol	2(g/lit) + 6(ml/lit)
T_{5-C}	Gelatin + Silicasol	2(g/lit) + 7(ml/lit)
T_{6-A}	Gelatin + Bentonit + Silicasol	2(g/lit) + 4(g/lit) + 7(ml/lit)
T_{6-B}	Gelatin + Bentonit + Silicasol	2(g/lit) + 5(g/lit) + 6(ml/lit)
T_{6-C}	Gelatin + Bentonit + Silicasol	2(g/lit) + 6(g/lit) + 5(ml/lit)

[a]all treatments done in three replicate

GJCS reduces acidity and eliminates materials blurring the grape juice. The soil was first dissolved into part of grape juice and then added to the samples and was thoroughly mixed. After 2-3 hours, grape juice concentrate cracks on the surface. At this time the existing foam should be removed from the surface and sieved through a piece of percale. In all other treatments, the acidity of grape juice was set off by calcium carbonate (42.5 g/5 liters of grape juice) to the final pH=8.5. Then the clarifying agents were added and the juice was sieved by a piece of percale after 30 minutes. All samples were finally transferred to the cooking section and concentrated to brix=70±2.

Test procedure of metals.

After homogenizing grape juice concentrate sample, two grams of the homogenized sample was weighed inside a crucible, washed with acid (acid wash: All laboratory glassy kits used in this test, were put in Nitric Acid of 10% - volumetric-volumetric for the entire one night and then washed using distilled water). After washing, the glassy kits were dried in oven – (Memmert-Germany) - at 80 centigrade degrees for one hour). The crucible was put in oven for 1-2 hour/s at 100 centigrade degrees and eventually, water in the said sample evaporated. Then, the said crucible was put on fire beneath Hood until respective sample was completely burnt with no smoke. The said crucible was transferred to an electric furnace (Barnstead Thermolyne F6000, Germany). The said furnace was regulated at 450 centigrade degrees. The crucible was removed from the furnace (6-8 hours) and became cool. Then, the resulted white ash was solved in Nitric Acid of 1 Molar, using a volumetric flask and clarified accordingly. The prepared solution, using the said sample, together with Blank (Nitric Acid – 1 Molar) and respective standards, was put in flame atomic absorption spectrophotometry device (Perkin Elmer, America) in order to measure their optic absorption. Absorbed quantities have changed to concentration using Calibration Curve. Final concentration of metals in the said sample has been obtained by inserting dilution coefficient[3].

Statistical analysis

The design of experiment used was random complete blocks (factorial) with two factors (type and quantity of clarifiers) and three repetitions. Results were statistically analyzed, using the MSTAT-C software and

ANOVA test. The medians were compared through LSD test at $p< 0.05$.

RESULTS AND DISCUSSION

Analysis of grape juice

Analysis of grape juice samples for pH and acidity showed these parameters respectively equal to 3.56±0.01 and 0.59±0.01. The mean value for brix of grape juice samples was 23.1±0.37 which increased to 71.1–72.8 after concentration using different clarifiers.

The effect of type and quantity of clarifying agents on quantities of iron, copper, zinc, calcium and magnesium have been given in TABLES 2, 3, 4, 5 and 6. ANOVA test showed that there is a significant difference between different treatments in the view of quantity of minerals at statistical level of 5% ($P<0.05$). It should be noted that all tests have been repeated for three times. Respective figures given in the aforesaid tables are the average of three repetitions.

Considering comparison of averages, the results show that maximum quantity of iron and copper has been obtained from Gelatin-Bentonit-Silicasol treatment and maximum quantity of zinc and magnesium has been obtained from Gelatin-Bentonit treatment and maximum amount of calcium has been obtained from Gelatin-Silicasol treatment. The least quantity of iron and calcium has been obtained from Bentonit treatment, the least quantity of copper and zinc has been obtained from syrup soil treatment and the least quantity of magnesium has been obtained from Silicasol treatment. Increase of syrup soil except for calcium and magnesium leads to increase of other metals, measured. While bentonit treatment, increase of the quantity of bentonit has significantly increased iron and zinc. However, it has had no effect on quantity of copper, calcium and zinc. While Silicasol treatment, increase of its quantity of Silicasol has led to significant increase of that of iron, copper and zinc. However, it has had no significant effect on the quantity of calcium and magnesium. Gelatin-Bentonit treatment, increase of quantity of Bentonit on iron, copper, zinc and magnesium has not been significant. However, it has significantly decreased the quantity of calcium. In Gelatin-Silicasol treatment, increase of the quantity of Silicasol has increased that of copper and zinc and decreased quantity of calcium. However, it has had no significant effect on quantity of iron and magnesium. While Gelatin-Bentonit-Silicasol treatment, increase of the quantity of Bentonit and Silicasol has led to increase of copper and zinc. However, it has had no significant effect on quantity of iron, calcium and magnesium. Studying the resources in measurement of metals in grape juice concentrate with various clarifier materials, no researches have been obtained. However, study of references and researches with respect to measurement of the quantity of metals in grape juice concentrate, produced using a traditional method, is conducted and in concluding, the results obtained from this research are compared to those of other researches and discussed accordingly.

TABLE 2 : Effect of type and quantity of clarifiers on iron

Treatments	Clarifier concentration		
	A	B	C
T_1	$94.8±1.71^{i}$	$106.11±2.46^{h}$	$111.72±2.21^{g}$
T_2	$65.01±1.16^{l}$	$79.25±2.32^{k}$	$88.97±2.35^{j}$
T_3	$107.5±3.11^{h}$	$113.96±2.84^{g}$	$124.87±1.52^{d}$
T_4	$123.04±1.57^{def}$	$124.72±1.68^{de}$	$136.59±2.00^{c}$
T_5	$121.28±2.39^{ef}$	$138.15±1.63^{bc}$	$141.33±1.67^{ab}$
T_6	$120.1±2.58^{f}$	$141.31±2.08^{ab}$	$144.70±1.42^{a}$

Different letters on data differ significantly (P<0.05, n=3), Different treatments refer to TABLE 1

TABLE 3 : Effect of type and quantity of clarifiers on copper

Treatments	Clarifier concentration		
	A	B	C
T_1	$35.95±0.85^{h}$	$44.56±1.31^{g}$	$47.23±1.36^{f}$
T_2	$43.25±0.76^{g}$	$55.43±1.23^{c}$	$56.36±1.18^{bc}$
T_3	$28.08±0.36^{i}$	$52.8±1.30^{e}$	$55.34±1.24^{cd}$
T_4	$53.37±1.06^{de}$	$55.09±1.63^{cd}$	$58.38±1.65^{ab}$
T_5	$45.02±1.26^{g}$	$48.53±1.02^{f}$	$55.34±1.50^{cd}$
T_6	$53.35±0.97^{de}$	$57.66±1.59^{b}$	$59.85±0.92^{a}$

Different letters on data differ significantly (P<0.05, n=3), Different treatments refer to TABLE 1

TABLE 4 : Effect of type and quantity of clarifiers on Zinc

Treatments	Clarifier concentration		
	A	B	C
T_1	$36.00±0.87^{de}$	$42.06±0.99^{bc}$	$46.34±1.02^{def}$
T_2	$48.42±0.8^{bc}$	$57.59±1.74^{f}$	$60.85±1.22^{bc}$
T_3	$38.63±0.98^{bc}$	$48.45±0.83^{b}$	$58.78±1.77^{def}$
T_4	$57.4±1.61^{a}$	$58.51±1.24^{a}$	$61.37±1.98^{c}$
T_5	$38.45±0.43^{d}$	$45.24±1.41^{ef}$	$51.38±1.01^{bc}$
T_6	$51.25±1.09^{a}$	$59.72±1.4^{a}$	$62.32±1.88^{b}$

Different letters on data differ significantly (P<0.05, n=3), Different treatments refer to TABLE 1

TABLE 5 : Effect of type and quantity of clarifiers on calcium

Treatments	Clarifier concentration		
	A	B	C
T_1	123.13±1.61[ef]	106.64±2.78[j]	132.07±1.64[c]
T_2	110.85±2.64[i]	89.72±1.12[l]	124.94±1.67[e]
T_3	128.67±1.97[d]	106.36±2.47[j]	111.30±2.27[i]
T_4	124.04±2.72[e]	121.23±2.60[fg]	90.77±1.60[l]
T_5	135.41±1.27[a]	132.39±1.75[bc]	115.35±1.13[h]
T_6	93.10±1.04[k]	134.55±0.97[ab]	119.8±1.62[g]

Different letters on data differ significantly (P<0.05, n=3), Different treatments refer to TABLE 1

TABLE 6 : Effect of type and quantity of clarifiers on magnesium

Treatments	Clarifier concentration		
	A	B	C
T_1	96.36±2.32[f]	87.15±1.14[h]	115.07±1.49[b]
T_2	97.76±1.15[f]	105.72±1.90[de]	96.30±2.39[f]
T_3	88.19±1.66[h]	111.33±2.10[c]	95.35±1.58[fg]
T_4	120.69±2.80[a]	121.26±2.42[a]	92.13±1.08[g]
T_5	94.61±1.65[fg]	114.21±2.60[bc]	103.05±1.77[e]
T_6	112.07±1.90[bc]	85.14±1.19[h]	106.50±2.72[d]

Different letters on data differ significantly (P<0.05, n=3), Different treatments refer to TABLE 1

Demirozu et al.[11] conducted a study on 108 samples of grape juice concentrate, produced using a traditional method and reported the quantity of iron, copper and zinc in the said samples using GJCS within a range of 5.50-130 (with an average of 26.32), 0.06-18 (with an average of 2.90), 0.12-11.20 (with an average of 3.69) mg/kg. The results of our study indicate the quantity of iron, copper and zinc in the samples of grape juice concentrate in a range of 65.01-144.7 (with an average of 115.74), 28.08-59.85 (with an average of 50.31), and 36-62.32 (with an average of 51.26) mg/kg respectively.

Although the quantities of iron and zinc in our study have been more than the results obtained from the aforesaid researches, on a whole, one can say that the results lie in a low limit. However, with respect to the quantity of copper, the results obtained from our studies have been far more than those obtained from the said researches. Such variables as cultivation of grapes, varieties of grapes, geographical region and environment, type and compounds of soil of region for cultivation, and equipments used in production are the most significant factors affecting the quantity of elements in grape juice concentrate samples. Thus, the difference in various researches with respect to quantity of elements, subject of study, may be caused by the said factors. Because in this research, the most important goal is to study the effect of various clarifier elements on the quantity of metals in produced grape juice concentrate. Further to the aforesaid factors, on a whole, we can say that the main factor affecting the difference in quantity of the said elements in various treatments, type and compound as well as quantity of clarifying agents (Grape juice concentrate soil, Bentonit, Silicasol, Gelatin), used in producing grape juice concentrate. Type and quantity of additives using various clarifying agents with direct effect on pH of consuming grape juice play a great role in corrosion and oxidation of equipments used in producing grape juice concentrate. Such corrosion of the said equipments shall result in entrance of tiny metal filings in the fruit juice.

Ustun and Tosun[28] studied the quantity of metals in 11 samples of grape juice concentrate. The least and most quantity of respective parameters, studied in grape juice concentrate samples have been reported in detail as follows: Calcium: 50.86-206.13mg/100 g; Sodium 25.38-83.33 mg/100 g; Magnesium 11.03-68.31 mg/100g; Phosphorus: 0-95.06 mg/100 g; iron: 2.62-16.30 mg/100 g; copper: 0.29-0.94 mg/100 g; Zinc: 0.18-0.74 mg/100 g.

Artik and Velioglu[2] reported the quantity of the respective elements in grape juice concentrate as follows:

Sodium: 25.4-83.2 mg/100 g; Phosphorus: 81-95.06 mg/100 g; Potassium: 1470 mg/100g; Copper 0.29-0.94 mg/100g; Calcium 50.9-206/1 mg/100 g; manganese: 11.03-68.31 mg/100 g; Magnesium: 140 mg/100g; iron: 2.62-16.30 mg/100g; zinc 0.18-0.74 mg/100 g.

Batu[5] reported the quantity of Phosphorus in grape juice concentrate between 28.7-652.2 mg/kg. Karakaya and Artik[17] reported the quantity of iron in grape juice concentrate for 0.3 mg/kg while the quantity of iron (with an average of 115.74 mg/kg) in grape juice concentrate samples, subject of study far more than the said quantity.

Karakaya and Artik[17], Batu[5] reported the quantity of potassium in various syrups as 1.160 mg/kg and 1.359-2.874 mg/kg respectively.

Ozturk and Oner[21] reported the quantity of calcium and iron in grape juice concentrate as 0.084-

0.086% and 0.005-0.01% respectively. The results obtained from our studies, indicates the quantity of calcium (with an average of 116.68 mg/kg) and iron (with an average of 115.74 mg/kg) more in grape juice concentrate samples.

Zomorodi et al.[30], considering the effect of clarifying agents on the quality of grape juice concentrate, and through analysis of minerals, indicated that the effect of type of treatments on the quantity of such metals as calcium, zinc, manganese, copper and iron at level of 1% and on magnesium at level of 5% has been significant. Maximum quantity of magnesium and least quantity of other metals, which have been measured, were related to treatment of syrup soil. Increase of metal elements of fruit juice, especially when they are mixed with phosphate and complicated mineral compounds shall cause opacity of its color. Iron is combined with tannins of fruit juice, producing blue dye. More quantity of copper shall also cause opacity due to protein-tannin, especially during stock period of the fruit juice. The quantity of iron and copper, which naturally exist in fruit juice, doesn't cause opacity of the fruit juice. The average of quantity of iron and copper in grape is given as 30 and 6.5 mg per kg respectively. More quantity of iron and copper is associated with other resources rather than fruit. Using respective equipments, which are at risk of corrosion, shall lead to entrance of tiny metal filings at fruit juice. In order to prevent such oxidation, the equipments used must be resistant against oxidation and corrosion[13]. If metal ions of grape juice are separated using replacing cation resins, bright color of grape juice concentrate is kept during preservation period[10].

CONCLUSION

This research has been conducted in order to prevent the effect of various treats for clarifier on the quantity of minerals on grape juice concentrate. In consideration of comparison of averages, the results show that maximum quantity of iron and copper has been obtained from Gelatin-Bentonit-Silicasol treatment, maximum quantity of zinc and magnesium has been obtained from Gelatin-Bentonit treatment and maximum quantity of calcium has been obtained from treating Gelatin-Silicasol. The least quantity of iron and calcium has been obtained from treating Bentonit, the least quantity of copper and zinc has been obtained from GJCS treatment and the least quantity of magnesium has been obtained from Silicasol treatment. Such variable as manner of cultivation of grapes, varieties of grapes, environment, geographical region for cultivation and type and compounds of soil of the region, type and quantity as well as compounds of various clarifier materials, used in producing grape juice concentrate and equipment used in production are the most effective elements on quantity of elements of grape juice concentrate elements.

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