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Physicochemical properties and antioxidant activity of Doshab (a traditional concentrated grape juice)

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Abstract

Doshab is a concentrated and shelf-life extended form of grape juice. The present study was outlined to investigate the physicochemical properties including pH, titratable acidity, soluble solids (˚Brix), and water activity (a_w) as well as total phenolics and antioxidant activity of 19 Doshab samples from different production unites. The antioxidant activities of the samples were assessed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, β-Carotene bleaching and reducing power assays. The pH values and titratable acidity of Doshab samples were ranged from 5.3 to 6.2 and from 0.165 to 0.622 g per 100 g, respectively. In DPPH assay, radical scavenging activity of samples was ranged from 27.7% to 82.3%. Inhibition percentage (I %) of the linoleic acid oxidation was between 72.2% and 87.2%. Total phenolic contents of doshab samples were changed between 1.84 and 4.47 mg of gallic acid equivalent per g of the sample. There was no significant correlation between radical scavenging activity and total phenolic contents. A moderate correlation between I (%) and total phenolic content, as well as between reducing power and total phenolic content was obtained.

Introduction

Human body is continually exposed to free radicals which are implicated in pathogenesis of number of diseases including various cancers, cardiovascular diseases and diabetes (Cooper *et al*., 2004; Libby, 2006; Dembinska-Kiec *et al.*, 2008). Regular consumption of food containing natural antioxidants is expected to prevent the risk of many free radical-mediated diseases (Temple, 2000; Young and Woodside, 2001).

Grape (*Vitis vinifera*) is among the fruits with the highest concentration of antioxidant phenolic compounds (Iacopini *et al*., 2008). The health-promoting effects of grape and grape juice are believed to be related to the phenolic compounds (Zern *et al.*, 2005; Frederiksen *et al*., 2007). A wide range of biological activities of phenolic compounds such as anticarcinogenic, antiulceric, antiallergic, antiatherogenic, anti-inflammatory, antiarthritic, antimicrobial properties and antioxidant activity has been reported (Mangiapane *et al*., 1992; Liviero and Puglisi, 1994; Teissedre *et al.*, 1996; Siato *et al.*, 1998; Catterall *et al*., 2000; Terra *et al*., 2007).

Doshab is one of the Iranian traditional grape products which is produced through boiling and concentrating grape juice. The purpose of concentration is to extend the shelf-life by reducing the water content and to sterilize the grape juice. In first step of Doshab preparation, grapes were washed and crushed. Pressing the crushed grapes provides grape juice. The grape juice is then boiled with a calcareous substance called "Doshab soil" containing white soil which has high amounts of calcium carbonate. This soil reduces the acidity caused by naturally existing tartaric and malic acids by precipitating them as calcium tartarate and calcium malate, respectively. Furthermore, the soil acts as a clarifier agent because light particles ascend and heavy materials precipitate along with soil. Finally, grape juice is boiled in an open container (Tian) to obtain 65 - 78°Brix (Zomorrodi, 2005).

Recently there has been extensive research into the antioxidant activity of fruits and fruit juices (Szeto *et al*., 2002; Yau Yan *et al*., 2006; Dragovic´-Uzelac *et al*., 2007; Lim *et al*., 2007; Seeram *et al*., 2008; Strangeland *et al.*, 2009). However, there is no report on antioxidant potency of Doshab, Iranian traditional grape juice. The aims of this study were to investigate the physicochemical properties (pH, titratable acidity, °Brix and aw) as well as total phenolic contents and antioxidant activity of nineteen Doshab samples from different production unites.

Materials and Methods

Doshab samples

Nineteen different Doshab samples were obtained from different production unites around Urmia city and stored in a refrigerator until analysis. All samples

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were analyzed to determine the physicochemical characteristics (pH, titratable acidity, °Brix, a_w) and to measure total phenolics and their antioxidant activity. All analyses were performed in duplicate. The origin of all samples except sample 9 (mixture of red and white grape) were white grape.

Chemicals

Gallic acid, linoleic acid, β-carotene, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferric chloride and potassium ferricyanide ($K_3Fe(CN)_6$) were obtained from Chemical Co (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Analytical grade ethanol, methanol, Folin-Ciocalteu's phenol reagent, disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium carbonate, NaOH, and trichloroacetic acid were purchased from Merck (Darmstadt, Germany).

Physicochemical properties

The pH of Doshab samples were measured using a pH meter (E 520, Metrohm Herisau, Switzerland). Titratable acidity was determined by adding 5 g of doshab sample to 100 ml of distilled water and titrating with 0.1 N sodium hydroxide to pH of 8.4. The results were expressed as g tartaric acid/100 g (Haight and Gump, 1995). Total soluble solids were measured using a Hand refractometer (ERMA Inc., Tokyo, Japan). The a_w of samples were measured using a aw meter (novasina ms1-a_w, Axair Ltd., Switzerland).

Antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activities of the Doshab samples were measured using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) as described by Blois (1958) with some modifications. Fifty µl of sample solution (10% v/v) was added to 2 ml of methanol solution of DPPH (24 µg/ml). After shaking, the mixture was incubated at room temperature for 15 min in a dark place. Then, the absorbance was measured against a blank at 517 nm with a spectrophotometer. Radical scavenging activity (RSA) was calculated according to the following equation:

$$RSA\% = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

Where A_{blank} was the absorbance of control reaction (containing all reagents except the test compound), and A_{sample} was absorbance of test compound. BHT (1 mg/ml) was used as positive control.

Reducing power

The reducing power of the Doshab samples was determined according to the method of Oyaizu (1986). One ml of sample solution (10% v/v) was mixed with 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1 %). After incubation at 50˚C for 20 min, 2.5 ml of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 1500 g for 10 min. Finally, 2.5 ml of upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%). After 10 min, the absorbance was measured at 700 nm, against blanks that contained all reagents except the sample extracts. A higher absorbance indicates a higher reducing power. BHT (1 mg/ml) was used as positive control.

β-Carotene bleaching assay

The antioxidant activity of Doshab samples were evaluated according to the method described by Miraliakbari and Shahidi (2008) with slight modifications. Approximately 5 mg of β-carotene was dissolved in 10 ml chloroform. Then, 1 ml of this solution was pipetted to a round-bottomed flask containing 25 µL linoleic acid and 200 mg Tween 40. Chloroform was completely removed by rotary vacuum evaporator and 100 ml distilled water was added to residue and shaken vigorously to form an emulsion. A volume of 350 µl of each sample solution (10% v/v) was added to 2.5 ml of the above emulsion in the test tubes and incubated for up to 5 h at 50°C. After incubation, the absorbancies were measured at 470 nm. The antioxidant activity (inhibition percentage, I%) of the samples were calculated using the following formula:

$$I\% = (A_{\beta\text{-carotene after 5h assay}} / A_{\text{initial }\beta\text{-carotene}}) \times 100$$

Where $A_{\beta\text{-carotene after 5h}}$ assay was the absorbance values of β-carotene after 5 h assay and $A_{\text{initial }\beta\text{-carotene}}$ was the absorbance value of β-carotene at the beginning of the experiments. BHT (2 mg/ml) was used as positive control.

Assay for total phenolics

Total phenolic contents of the samples were determined using the Folin-Ciocalteu reagent assay (Singleton and Rossi, 1965), with gallic acid as standard. Briefly, 500 µl of the sample solution (10% v/v) was mixed with 2.25 ml distilled water and then 250 µl of Folin-Ciocalteu reagent was added. The mixture was vortexed for 30 sec and allowed to react for 5 min. Then, 2 ml of 7.5% Na_2CO_3 solution was

added. After incubation at room temperature for 120 min, absorbance of each mixture was read at 760 nm. The same procedure was also applied to the standard solution of gallic acid, and a standard curve was obtained. Total phenolic contents were expressed as mg of gallic acid equivalent per g (mg GAE/g) of the sample.

Results and Discussion

Physicochemical properties

Physicochemical characteristics of Doshab samples are given in Table 1. pH values and titratable acidity of Doshab samples were ranged from 5.3 to 6.2 and from 0.165 to 0.622 g per 100 g, respectively. The total soluble solids and aw of the samples were ranged from 65 to 81°Brix and from 0.51 to 0.8, respectively. Higher pH value is due to addition of the soil to grape juice. This soil has high amounts of $CaCO_3$ which reacts with tartaric and malic acids. Therefore, the acidity is decreased due to precipitate of these acids. A wide range of °Brix can be attributed to different boiling time used in different production unites.

Antioxidant activity

The antioxidant activity of the samples was evaluated using three separate methods, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, beta carotene bleaching and reducing power assay.

DPPH radical scavenging assay

It was reported that grape juice possesses antioxidant properties (Vinson *et al*., 2001). There are different antioxidants present in plants and it is very difficult to measure each antioxidant component separately. Therefore, it is necessary that we use multiple assays for evaluating the antioxidant potential. In this study, the antioxidant potential of the samples was evaluated using three separate methods, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, beta carotene bleaching and reducing power assay. In DPPH assay, the hydrogen donating antioxidants react with the stable free radical i.e. DPPH˙ (deep violet color) and reduce it to DPPH with discoloration. The degree of discoloration indicates free radical scavenging capacities of the sample (Blois, 1958). Free radical scavenging activity of Doshab samples was measured by DPPH assay. As can be seen from Figure 1, the DPPH scavenging activity of samples was ranged from 27.7% to 82.3%. The sample No. 2 was the most effective in scavenging the DPPH free radicals.

Table 1. Physicochemical properties of different Doshab samples

Sample No	pH	Titratable acidity (g per 100 g)	a_w (25°)	°Brix
1	6.2	0.165	0.80	67.20
2	5.6	0.417	0.68	73.80
3	6.2	0.235	0.79	70.50
4	6.0	0.346	0.69	67.05
5	5.8	0.605	0.72	71.40
6	5.5	0.589	0.70	66.45
7	5.7	0.419	0.74	67.50
8	6.0	0.355	0.70	65.00
9	5.5	0.394	0.70	66.30
10	6.2	0.218	0.51	72.00
11	5.6	0.421	0.54	70.50
12	5.7	0.313	0.70	72.00
13	5.9	0.415	0.70	75.00
14	6.0	0.328	0.74	72.60
15	5.3	0.622	0.70	64.50
16	6.2	0.252	0.67	78.00
17	5.6	0.393	0.63	81.00
18	5.7	0.314	0.72	75.00
19	5.6	0.433	0.76	70.80

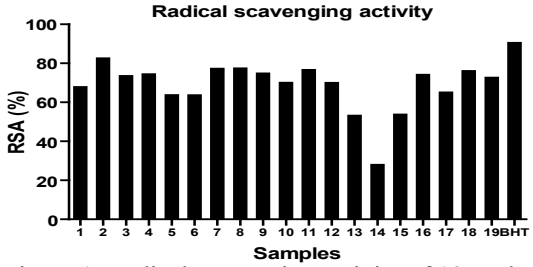

Figure 1. Radical scavenging activity of 19 analyzed Doshab samples

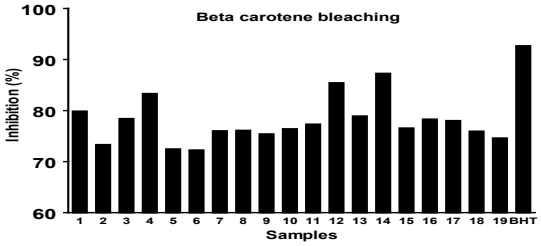

Figure 2. Inhibition percentage of the linoleic acid oxidation by 19 Doshab samples

β-Carotene bleaching assay

In β-Carotene bleaching assay, the yellow colour of β-Carotene was lost because of its reaction with radicals which are generated by linoleic acid oxidation in an emulsion. The presence of antioxidants can delay discoloration of β-carotene by neutralizing the free radicals (Kulisic *et al*., 2004). The potential of Doshab samples to inhibit lipid oxidation was evaluated using β-Carotene bleaching assay. The results of antioxidant activity of Doshab samples are presented in Figure 2. Sample No. 14 showed the highest inhibition percentage of the linoleic acid oxidation, while the antiradical activity of this sample, measured by DPPH method, was the lowest.

Reducing power

In reducing power assay, the yellow color of the test solution is changed to various shades of green and blue color depending upon the reducing capacity

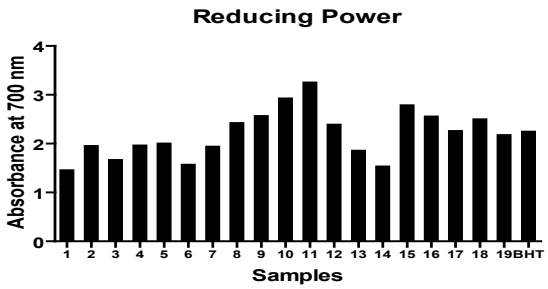

Figure 3. Reducing power of 19 analyzed Doshab samples

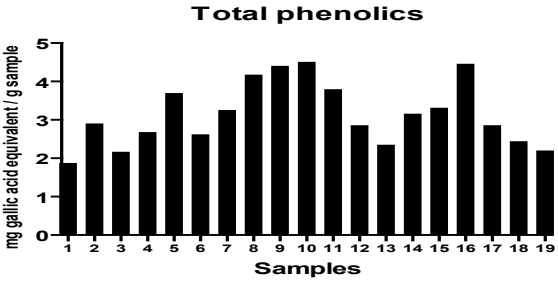

Figure 4. Total phenolics concentration in 19 Doshab samples

of each sample. Reducing capacities are generally associated with the presence of reductants in the antioxidant samples (Duh, 1998). Reductants cause reduction of the Fe^{3+}/ferricyanide complex to ferrous form. Therefore, the amount of ferrous complex can be monitored by measuring the formation of perl's Prussian blue at 700 nm (Gulcin *et al*., 2006). Increasing absorbance at 700 nm indicates an increase in reducing capacity. Figure 3 shows reducing power of Doshab samples. Sample No. 11 and 1 had the highest and the lowest reducing power, respectively.

Total phenolics

Total phenolic contents of the Doshab samples were determined by Folin-Ciocalteu method. Total phenolic contents of Doshab samples were changed between 1.84 and 4.47 mg gallic acid equivalent per g of sample (Figure 4). The mean value for total phenolic contents was 3.11 mg gallic acid equivalent per g. The higher concentrations of total phenolics were recorded in samples No. 10, 16, 9 and 8, respectively. It was also observed that samples No. 1, 3 and 19 had low concentrations of phenolic contents as compared with other samples. Folin-Ciocalteu procedure is a useful and rapid method for estimating phenolic content of plant extract (Luximon-Ramma *et al.*, 2003). Diversity in phenolic contents could be due to differences in Doshab processing operations, such as heating time and temperature as well as other factors related to grapes, such as grape maturity and cultivation practices.

A correlation between total phenolic content and antioxidant activity was done using the function CORREL from Microsoft Excel software. There was no significant correlation between the radical scavenging activity and the total phenolic content (R^2 = 0.047). However, a moderate correlation between I (%) of linoleic acid oxidation and total phenolics (R^2 = 0.42) as well as, between reducing power and total phenolic content was observed (R^2 = 0.61). Folin-Ciocalteu reagent reacts not only with phenolic compounds but also with other reducing compounds such as carotenoids, amino acids, sugars and vitamin C (Vinson *et al.*, 2001). In addition, the DPPH assay determines free antiradicals in food samples, while the Folin-Ciocalteu method determines both free phenolics and bound phenolics (Singleton *et al.*, 1999). In general, due to lake of data on antioxidant activity and total phenolics of Doshab, we could not compare the results of our present study with the literature.

Conclusion

Our study provides first data on physicochemical properties and antioxidant activity of Doshab. It is very difficult to assess the antioxidant activity of a product on the basis of a single method. The results obtained using three different methods to evaluate the antioxidant activity (DPPH, reducing power and β-Carotene bleaching assay) showed that Doshah is a good source of natural antioxidants. Since production units work with different processing factors (heating time and temperature), standardization of processing operations of Doshab is necessary. Further studies are needed to investigate composition, nutritional value, rheology, color and other characteristics of Doshab.

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