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Research Article

Determination of Antioxidant Power of Some Various Grape Juices by Voltammetric Method and Its Correlation with Polyphenolic Content

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ABSTRACT

Objective: Due to rich resources of phenolic components such as anthocyanin and flavonoids, grape has grabbed lots of attention. Antioxidants are the frontline of the human immune system against free radicals and they are of high importance for the human health. **Methods:** In order to determine antioxidant activities, the amount of phenols, anthocyanin and ascorbic acid of 2 grapes' varieties ("white seedless" and "red seedless" as two famous varieties of Qazvin's grapes) have been studied with cyclic voltammetry including, DPPH and Folin-Ciocalteu reagent at room and refrigerator temperature, before and after pasteurization process. **Results:** The results of this study has shown that the two varieties of grapes did not have a meaningful difference in total antioxidant concentration; and cyclic voltammetry method has been more or less able to define the type of antioxidant according to the results of this study. Moreover, the two types of grapes had a significant difference in concentration of ascorbic acid and anthocyanin so that concentration of ascorbic acid in white grape juice in room temperature was 53ppm while at the same situation it was 24ppm in red grape juice.

1.INTRODUCTION

Grape "Vitis Vinifera" is a species of Vitis from "Vitaceae" family. Nowadays, Grape is one of the significant commercial products of temperate climate and its production is increasing particularly in Asia (Valamoti and Mangafa, 2004; Ercisli and

Orhan 2007). Beside the commercial value of the Grape, its nutritional values, natural colors and a wide range of antioxidant ingredients are highly admired (Hulya, 2007). Antioxidants are a vital compound which can defend human's body against the free radicals and oxidative stress. Antioxidants are range of vitamins, organic compounds and

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enzymes which can protect human body's cells and tissues from the harmful effects of free radicals. Antioxidants as phenolic compounds can be found in different parts of the plant. Since they have an important effect on the quality of the grape fruit including its smell, taste, bitterness and the astringent taste, their amount and activities are highly regarded (Benvenuti *et al.*, 2002). The total phenol of the grape's fruit can be different because of plant's variety, soil composition, storage condition, weather and geographical situation (Hernandez-Jimenez *et al.*, 2009). Phenol compounds are mostly including anthocyanin, proanthocyanins, flavonols, flavonoids and phenolic acids (Prior *et al.*, 1998). Presently there are evidences that show the juices have nutrients and fruitful compounds which are comparable with fruits (Mullen *et al.*, 2007) and they can increase ailments and can also hold up start of Alzheimer diseases (Dai *et al.*, 2006). In the past few years, because of high antioxidants sources (phenols and anthocyanin), the importance of red fruits has increased (Salehi *et al.*, 2013). In a research in Iran on some of the Fars Province grapes varieties, antioxidant activity and its correlation with phenol and anthocyanin amount have been studied. According to the research there is a strong link between antioxidant activity and amount of anthocyanin. It is also found that there are a various factors like latitude, storage conditions and especially variety of the cultivar having effect on the amount of anthocyanin (Salehi *et al.*, 2013). The total phenolic content and antioxidant activity of some red wine by Pulse techniques, HPLC and spectrophotometry were investigated in some other studies and it observed that the difference of differential pulse is a sensitive and selective method which has a considerable importance for determination of total phenol (Walkowiak-Tomczak, 2007; Seruge *et al.*, 2011). In another research, the antioxidant activity changes in a kind of berry juice which was pasteurized during storage was studied and it observed that the process of high thermal makes high stability or increase the antioxidant activity (Genova *et al.*, 2012). Therefore, the purpose of this research is to study the effect of storage conditions (time and temperature) on the antioxidant activity and polyphenol compounds in white and red seedless grapes of Takestan region (Qazvin Province in Iran).

2. MATERIALS AND METHODS

2.1. Sample and reagents

Two varieties of seedless grapes including white and red were provided from Takestan Region. DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent from Sigma, Folin-Ciocalteu reagent, mono hydrated gallic acid, methanol, inert salt (NaCl, KCl), sodium carbonate and the buffers all has been provided from Merck.

2.2. Preparation of grape juice

After harvesting the fruits (white and red seedless grape) late September, they transferred to Zanjan University laboratory. They stored for one day in 4° C. After the tail cutting and washing of the grapes with pure water, juice was extracted by commercial centrifugal juicer from "Pars Khazar".

2.3. Grape juice pasteurization

Pasteurization was performed by the associated laboratory of "Pastor Institute" on the juice. Since the heat exchanger plate was used, Juice was pasteurized at the temperature of 90-92° C for 1 min and then cooled down to the temperature of 30-35° C and then packed in 200-gram polyethylene cup.

After placing the samples (including both white and red grape juice and having two types of pasteurized and unpasteurized for each one) in two temperatures in (4° C) and the ambient temperature (22 °C) and white grape juice was used as an index. Respectively in different time periods including same day, one day after, fifth day after, fourteenth day after and thirtieth day after, the following tests were performed.

2.4. Cyclic voltammetry

In this study, a three-electrode system was used. The system consists of a working electrode, a reference electrode and a counter electrode. These types of systems are the most common used methods in electrochemical tests and especially cyclic voltammetry. In all of the experiments in this study, the silver chloride electrode as the reference electrode has been used. The working electrode used in this project was made of glassy carbon. In this research we also used platinum as the counter electrode with a height of 1 cm and a diameter of 5.0 mm. The solution used in this experiment was

prepared by adding affectless salt (NaCl) to grape juice (Tawirirana and Ozoemena, 2009).

2.5. Measuring the amount of vitamin C

In this research the amount of ascorbic acid was studied by cyclic voltammetry (Mazumdar and Majumder, 2003).

2.6. DPPH method

To perform this experiment, the method of Lin and Tang was used (Lin and Tang, 2007). At the first a 2 to 10 ml of standard solution of gallic acid with 15 ml of 1/0 mM DPPH reagent mixture was placed in the dark for 30 minutes. Then absorbance was measured by a spectrophotometer at 517 nm. (Absorption control). Further a 5 ml sample of grape juice diluted with 15 mL of DPPH reagent mixture and reached to a volume of 25 and kept in the darkness for 30 minutes. In the next step, time of the absorbance was measured at a wavelength of 517-515 nm by spectrophotometer (Equation 1). (Absorption Sample).

Eq. (1):

$$\% \text{inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} * 100$$

It should be mentioned that in the "control sample", the extract should be replaced by methanol and finally the percent of inhibition of DPPH radical in the extract should be calculated.

2.7. Folin-Ciocalteu measurement

Total phenols by Folin-Ciocalteu reagent were measured. To perform this experiment, the National Standard of Iran No. 8986 was used (Institute of Standards and Industrial Research of Iran, 2006). Standard curve were made using mono hydrate gallic acid and total phenol was calculated based on the standard curve.

2.8. Statistical Analysis

The experimental design was based on completely randomized block design with three repetitions for each figure. The effect of the time was considered as

the block and for the analysis Microsoft Excel and Matlab software were used.

3. Results

The results of cyclic voltammetry showed that this method can determine density and almost type of antioxidants in addition to antioxidant activity.

Figure 1, shows two Voltammograms which "A" is related to white grape juice on the first day and after adding NaCl and Voltammogram "B" is shown the gallic acid with concentration of 300ppm, the figure indicates that the grape juice and gallic acid with concentration of 300 ppm are comparable. By this figure, antioxidant activity and concentration in the two samples of grape juice and in the two ambient and refrigerator temperature has been studied.

Ascorbic acid in amounts of 50 ppm has been used to determine type of compound which is oxidized before of gallic acid (peak A₁) (since the peak of oxidation of ascorbic acid is very low) And in a same potential, the current increased, which indicates that based on oxidation potential the cyclic voltammetry can determine type of antioxidants. (Figure 2) Also the effect of temperature on concentration and antioxidant activity were studied. It is observed that the currents are proportional to the concentration of antioxidants. In the A₁ area it is dropped with a very fast process at ambient temperature to the refrigerator temperature. The A₂ peak is for anthocyanins and has a greater oxidation potential than the A₁ peak. The A₃ peak is related to other phenolic compounds that the anodic peak potential area is greater than the other compounds and it is more difficult to oxidize and also an increase in current is observed in the refrigerator temperature in the area of A₃.

This could be because of the reason that some of the antioxidants related to the area of A₁ and A₂ after the oxidation and degradation and after some time can form the phenolic or acidic derivatives (carboxylic acids, aldehydes) that these compounds can again show the same behavior of antioxidant (Table 1, 2) (Talcott et al., 2003; Ozgen et al., 2007, Lin and Tang, 2007). And also the effect of temperature over time on concentration and antioxidant activity of red grape juice were studied. By comparing the antioxidant activity of these two

types of grapes at a same temperature (ambient or refrigerated), no significant differences were observed but by comparing the antioxidant activity in the non-same temperature (ambient and refrigerated) there was a significant difference between the two storage temperatures (Table 3).

3.2. Study of effect of pasteurization during the storing on antioxidant activity of pasteurized white grape juice by Cyclic voltammetry

With Comparing of the unpasteurized juice grapes with pasteurized one, it was clearly observed that the pasteurization process at the first reduces the concentration and activity of antioxidants but increases their stability during storing.

This could be because the reason that the Pasteurization can break chemical bonds of polyphenols and as a result the soluble phenol with low molecular weight releases. It can increase the phenolic compounds and these compounds consequently can increase stability and antioxidant activity which also have a positive correlation between antioxidant activity and phenolic compounds. It is also observed that in pasteurized grape juice during storage at refrigerator temperature the total antioxidant activity is maintained better than the ambient temperature (Nicoli *et al.*, 1999; Talcott *et al.*, 2000; Walkowiak-Tomczak, 2007).

3.3. Folin-Ciocalteu measurement

A comparison of the total antioxidant activity and total phenolic compounds in white grape juice stored at refrigerator temperature using both Folin and Cyclic voltammetry was carried out and the Folin method also showed almost the same results related to the changes of concentration of antioxidants in white grapes. For example, after the first day using the cyclic voltammetry method, the antioxidant activity was 83% and in the same day, with Folin method it was about 81% which shows a relatively equal amount of antioxidants.

3.4. Antioxidant activity measurements

A comparison of the total antioxidant activity with cyclic voltammetry and DPPH assay on white grape juice stored at refrigerator temperature was performed. The results (both of the initial concentration and decreasing) of using DPPH -as a well-known and accepted method- showed a good correlation with voltammetry data. For example, the initial concentration of antioxidants in DPPH assay and voltammetry concentrations was 674 ppm and 670/7 ppm which showed the accuracy of the data and also approved voltammetry method. According to a statistical study it can be expressed that the results obtained by this method comparing with cyclic voltammetry method -with a confidence level of 95%- are compatible with each other (Amidi *et al.*, 2011).

Table 1:

Concentration of antioxidant compounds in white grape juice during storage at ambient temperature in ppm.

Day	Total antioxidant activity	A ₁	A ₂	A ₃
0	670/6	53/39	380/08	226/34
1	551/64	36/22	300/7	200/3
5	389/08	18/28	169/99	193/61
14	284/48	5/27	62/06	186/41
30	112/76	1/55	17/29	101/01

Table 2:

Concentration of antioxidant compounds in white grape juice during storage at refrigerator temperature in ppm.

Day	Total antioxidant activity	A ₁	A ₂	A ₃
0	670/76	52/31	376/05	221/51
1	558/71	34	306/04	205/14
5	459/02	24/7	253/65	163/85
14	354/86	17/96	175/94	153/81
30	289/95	13/72	102/24	172/41

Table 3:

Concentration of antioxidant compounds found in pasteurized white grape juice during storage at ambient temperature in ppm.

Day	Total antioxidant activity	A ₁	A ₂	A ₃
0	612/46	44/58	336/82	213/32
1	539/36	30/4	318/41	178/36
5	423/68	24/11	214/4	177/24
14	278/16	8/75	105/43	181/89

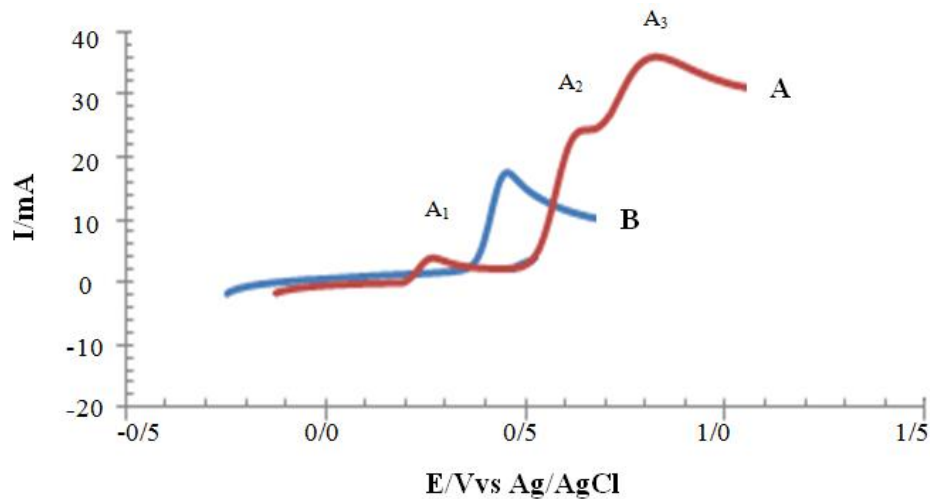


Figure. 1. Voltammogram A related to white grape juice and Voltammogram B related to gallic acid.

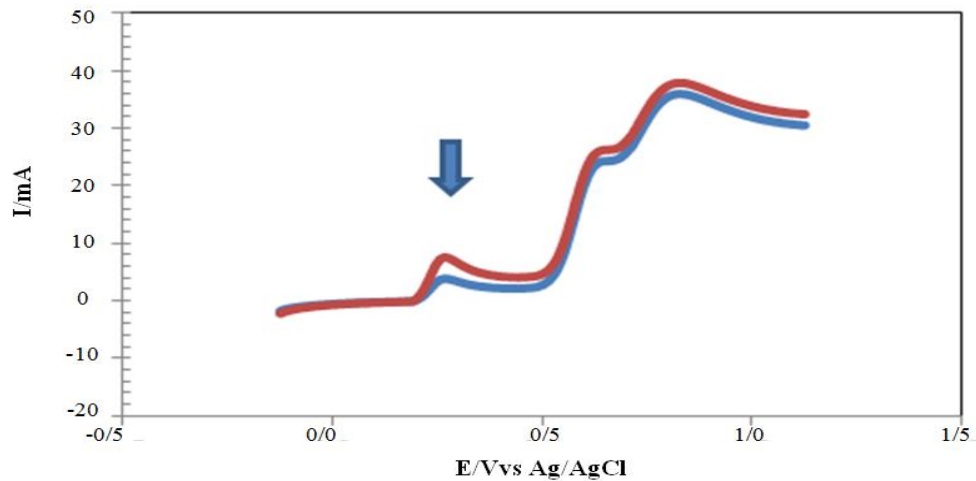


Figure. 2.

Voltammogram A related to white grape juice before adding ascorbic acid and Voltammogram B after adding ascorbic acid.

CONCLUSION

1- The voltammetry method for determination and calculation of the amount of antioxidants in samples of grape juice has a high features and it is comparable to the standard method of DPPH and Folin-Ciocalteu's method.

2- Considering the amount of antioxidants and voltammetry method detection limitation, this method is capable for detecting different kinds of grape antioxidants in the grape juice samples based on the oxidation potential. It also can be used to investigate the concentration changes.

3- Due to the comparatively simple application and the non-use of various chemical reagents and also the derivation methods (in the voltammetry method to produce ionic conductivity it is needed to add salt to the environment only), This method can be a good alternative to the method of derivation based absorptiometry.

4- Based on the results obtained from different methods, it can be said that the two samples studied

grapes (*Vitis Vinifera*.L.var.Bidane Sefid and *Vitis Vinifera*.L.var. Bidane Qermez) despite their color differences, do not have much differences in the amount of antioxidants.

5- Conditions (temperature) of storage of samples have a significant impact on the both process of concentration and antioxidant activity of grape juice. Even after a day of squeezing, samples stored at ambient temperature have a greater decrease of concentration.

6- In addition to temperature, Pasteurization also has a significant role in changing the activity and concentration of antioxidants and it can be said that the pasteurized samples are facing with a slower destruction of antioxidants.

7- The results also showed that probably due to the high temperature used in pasteurization process, reduce the initial concentration of antioxidants happens.

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