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Evaluation of the antiradical and antioxidant potential of grape extracts

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Abstract

Grape seed and bagasse extract obtained from Narince grape cultivar using different solvent mixtures were assayed for their antioxidant properties. Total phenolic contents of the extracts were determined by the Folin-Ciocalteu method. Antioxidant activities of the extracts at different concentrations were evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, hydrogen peroxide scavenging and phosphomolybdenum methods. Also the extracts, as natural antioxidants, were assayed during eight weeks storage of refined poppy oil at 70 °C. For this reason peroxide value was used as a criterion to assess the antioxidant activity of grape extracts.

The grape seed extracts showed strong antioxidant activity, by measuring their capacity to scavenge DPPH and hydrogen peroxide; to reduce Mo(VI) to Mo (V) and to decrease in the rate of peroxide formation, when compared to bagasse extract. Antioxidant activities of the extracts increased when the extract concentration increased.

Keywords: Grape seed extract; Grape bagasse extract; Total phenolic content; Antioxidant activity

1. Introduction

Lipid peroxidation is one of the main reasons for deterioration of food products during processing and storage. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) are widely used as food additives to increase the shelf life, especially of lipids and lipid-containing products by retarding the process of lipid peroxidation. However, BHT and BHA are known to have not only toxic and carcinogenic effects on humans (Ito et al., 1986; Wichi, 1988), but also abnormal effects on enzyme systems (Inatani, Nakatani, & Fuwa, 1983). Therefore, the interest in natural antioxidants, especially of plant origin, has greatly increased in recent years (Jayaprakasha & Jaganmohan Rao, 2000). Natural antioxidants can protect the human body free radicals that may cause some chronic diseases including cancer, cardiovascular diseases and cataract (Kinsella, Frankel, German, & Kanner, 1993; Lai, Chou, & Chao, 2001). The researches conducted on the antioxidant activities of some plants as natural antioxidants generally focused on the herbs and aromatic plants (Gülçin, Şat, Beydemir, Elmastaş, & Küfrevioğlu, 2004; Lu & Foo, 2001; Miliauskas, Venskutonis, & van Beek, 2004; Pizzale, Bortolomeazzi, Vichi, Überegger, & Lanfranco, 2002; Zheng & Wang, 2001).

The antioxidant properties of plant extracts have been attributed to their polyphenol contents (Lu & Foo, 2001; Murthy, Singh, & Jayaprakasha, 2002; Revilla & Ryan, 2000). So plants containing high-level of polyphenols have a great importance as natural antioxidants. Many byproducts and wastes generated by agroindustries contain polyphenols with potential application as food antioxidants and preventive agents against some diseases (Torres et al., 2002). And it is well known that the grape skins, seeds and stems, waste products generated during wine and grape juice processing, are rich sources of polyphenols

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(Macheix, Fleuriet, & Billot, 1990; Murthy et al., 2002; Saito, Hosoyoma, Ariga, Kataoka, & Yamaji, 1998).

The aims of this study were to determine the antiradical and antioxidant activities of grape seed and bagasse extract from different mixed solvent extractions and to determine the total phenolic compounds of the extracts to find out the relationship between antioxidant activities and total phenolic content. The other objective of this study is to investigate the utilization of the extracts as an alternative natural antioxidant to synthetics in poppy oil.

2. Materials and methods

Grapes of one of the most popular white wine-making grape cultivars grown in Turkey, Narince, were collected at optimal maturity from the experimental vineyard of the Agricultural Faculty of Ankara University (Ankara, Turkey).

2.1. Extraction

After harvest, undamaged and disease-free berries were snipped from clusters. After seeds were manually separated from whole berries, seeds and bagasse (berry without seed and juice) were dried at room temperature, separately. Dried grape seeds and bagasse were crushed in a grinder for 2 min, but at 15 s intervals the process was stopped for 15 s to avoid heating the sample. In order to remove the fatty materials from seeds, the powdered grape seeds (100 g) were extracted in a Soxhlet extractor for 6 h with 150 ml of petroleum ether at 60 °C. The defatted grape seed powder was re-extracted in a Soxhlet apparatus for 8 h with 200 ml of acetone:water:acetic acid (90:9.5:0.5) at 60 °C (grape seed extract 1, GSE 1) as described by Jayaprakasha, Selvi, and Sakariah (2003). In another extraction for seeds, ethyl acetate:methanol:water (60:30:10) at 60 °C (grape seed extract 2, GSE 2) was used instead. The powdered bagasse (100 g) was extracted in a Soxhlet apparatus for 8 h with 200 ml of ethyl acetate:methanol:water (60:30:10) at 60 °C (grape bagasse extract, GBE). The extracts were concentrated by rotary evaporation under vacuum at 70 °C to get crude extracts. The extracts were stored in a desiccator until use.

2.2. Determination of total phenolic content

Total phenolic contents of the extracts were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965), calibrating against catechin standards and expressing the results as mg catechin equivalents (CAE)/g extract. Data presented are average of three measurements.

2.3. Determination of antiradical activity

The free radical scavenging activity of extracts were examined by comparing to those of known antioxidants such as BHT, BHA and rutin by 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method of Lee et al. (1998). Briefly, a 1.0 ml solution of the extracts at different concentrations (25, 50 and 100 μg/ml) in methanol was mixed with 2.0 ml of methanolic solution of DPPH (10 mg/l). The mixture was shaken vigorously and allowed to stand at room temperature for 5 min. Then the absorbance was measured at 517 nm against methanol as the blank in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

The percent of DPPH decoloration of the samples was calculated according to the formula

$$\text{antiradical activity} = 100 \times (1 - \text{absorbance of sample}/\text{absorbance of control}).$$

All determinations were done in triplicate.

2.4. Determination of antioxidant activity

The antioxidant activities of extracts were evaluated by the formation of phosphomolybdenum complex method according to Prieto, Pineda, and Aguilar (1999). Briefly, An aliquot of 0.4 ml of sample solution (100 μg/ml in methanol) was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The blank solution contained 4 ml of reagent solution and the 1 ml of methanol. The vials were capped and incubated in a water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid.

All determinations were done in triplicate.

2.5. Determination of hydrogen peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch, Cheng, and Klauning (1989). A 2 mM solution of hydrogen peroxide was prepared in phosphate buffer (pH = 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm. Extracts (10, 25 and 100 μg/ml) in distilled water were added to 0.6 ml of hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing in phosphate buffer without hydrogen peroxide.

The percent of scavenging of hydrogen peroxide of extracts and standard compounds (BHA and BHT) was calculated according to the formula

$$\%\ \text{scavenged}\ H_2O_2 = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the samples of grape extracts and standards.

All determinations were done in triplicate.

2.6. Peroxide value

The antioxidant activity of the extracts was tested on poppy oil and expressed as the decrease in the rate of peroxide formation. Refined poppy oil, free of additives, was used as the substrate for oxidation studies. Poppy oil used in this study mainly comprised 71.3% linoleic, 16.5% oleic, 9.0% palmitic and 2.7% stearic acid. Fatty acid composition of poppy oil was determined according to the method of Baydar, Marquard, and Turgut (1999). And also its peroxide value was found as 2.5 meq/kg.

Two grams of oil was accurately weighted and the extracts were added directly to the oil at a concentration of 2 mg/ml (0.2 wt.%). BHA and BHT were mixed in oil for a comparative study at their legal limit of 0.2 mg/ml (0.02 wt.%) (Duh & Yen, 1997). Control samples of poppy oil without antioxidant were also placed under same conditions. All samples were incubated in 10×100 mm open beakers at 70 °C in the dark. The peroxide values of the samples were determined at one-week intervals according to the method of the American Oil Chemists' Society (AOAC, 1990). All determinations were done in triplicate.

3. Results and discussion

The yields and total phenolic contents of grape seed and bagasse extract with different solvent mixtures are given in Table 1. Yields of the extracts ranged from 16.2% to 6.2% and the total phenolic contents varied from 704 to 24 mg CAE/g. Grape seed extracts had higher extract yields and total phenolic contents than the bagasse extract. While the highest extract yield was obtained from the GSE 2 extracted with ethyl acetate:methanol:water (60:30:10), the maximum total phenolic content was found in the GSE 1 extracted with acetone:water:acetic acid (90:9.5:0.5). Murthy et al. (2002) and Jayaprakasha, Selvi and Sakariah (2003) reported that different amounts of total phenolic contents were found in different solvent extracts of grape seeds and pomace. On the other hand not only the amount of the extract yield but also the total phenolic content was very low in bagasse extract. The amounts of total phenolic contents of seed extracts in the present study were similar to the amounts reported by findings of Parejo et al. (2002) and Göktürk Baydar, Sağdıç, Özkan, and Çetin (2006).

Radical scavenging activities of grape extracts and standards at different concentrations were tested by the DPPH method and the results are shown in Fig. 1.

Table 1
Yield and total phenolic content of grape seed and bagasse extract

Extract	Yield (%)	Total phenolic content (mg CAE/g)
GSE 1	15.3 ± 2.3	704 ± 42.2
GSE 2	16.2 ± 2.8	671 ± 54.7
GBE	6.2 ± 1.0	24 ± 4.3

Values expressed are mean ± SD of three experiments.

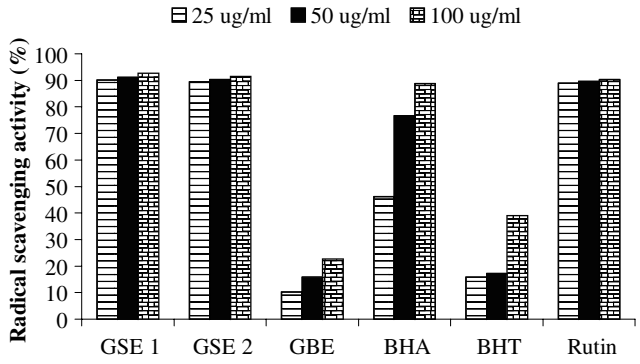

Fig. 1. Radical scavenging activities of extracts and standards at different concentrations.

DPPH scavenging activity increased in the following order: GBE < BHT < BHA < rutin < GSE 2 < GSE 1. The radical scavenging activities of the seed extracts were considerably better than that of bagasse extract. Grape seed extracts almost completely inhibited DPPH absorption. GSE 1 at 25, 50 and 100 μg/ml was the most effective DPPH radical scavenger with the inhibition of 90.2%, 91.1%, 92.6%, respectively. The average DPPH radical scavenger values for GSE 2 at 25, 50 and 100 μg/ml were 89.5%, 90.3% and 91.6%, respectively. On the other hand, bagasse extract contained remarkably lower amounts of radical scavenging compounds. Although scavenging activity of bagasse extract on DPPH radicals increased from 25 μg/ml to 100 μg/ml, at 100 μg/ml radical scavenging activity was only 22.7% for GBE. Rutin at all concentrations showed almost the same radical scavenging activity with the grape seed extracts, while BHT exhibited weak to moderate activity compared to grape seed extracts. When the concentration increased, a regular increase was observed in the scavenging activities of BHA. At 25 and 50 μg/ml, BHA showed lower activities compared to the grape seed extracts, but at 100 μg/ml the scavenging activity of BHA reached to those of seeds extracts with very close values. Similarly, Parejo et al. (2002) found that grape seed extract exhibited the highest free radical scavenging activity compared to BHA.

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods (Gülçin et al., 2004). The addition of the extracts to the DPPH solution caused a rapid decrease in the optical density at 517 nm. The degrees of discoloration indicate the scavenging capacity of the extract. Free radicals cause autoxidation of unsaturated lipids in food (Kaur & Perkins, 1991). The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity (Baumann, Wurm, & Bruchhausen, 1979). Antioxidants cease the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups. Therefore formed stable end-product does not permit further oxidation of the lipid (Sherwin, 1978).

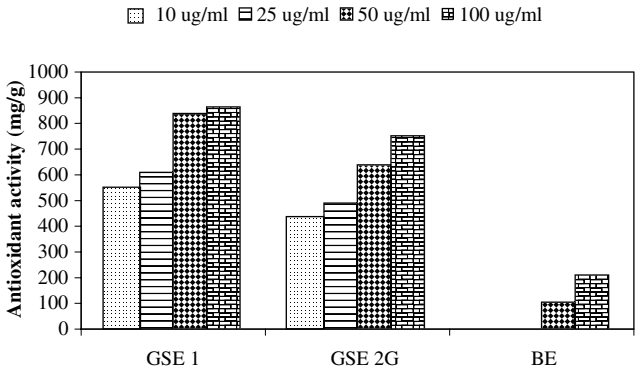

Fig. 2. Antioxidant capacity by phosphomolybdenum method (mg/g) of grape extracts at different concentrations.

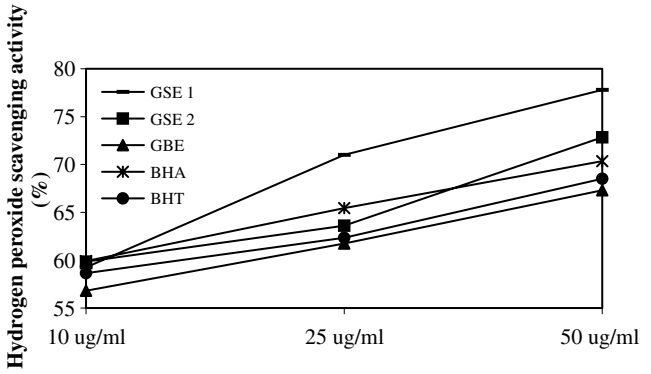

Fig. 3. Hydrogen peroxide scavenging activities of grape extracts and standards at different concentrations.

The antioxidant activity of grape extracts at different concentrations as measured by phosphomolybdenum method is presented in Fig. 2. It can be seen that grape extracts at different concentrations exhibited various degrees of antioxidant activity. Parallel to the increase in the extract concentration, antioxidant activity of the extracts increased. Both grape seed extracts posses more antioxidant activity than bagasse extract in the phosphomolybdenum method. The results showed that GSE 1 exhibited antioxidant activity ranging from 863 to 125 mg/g extract (equivalent to ascorbic acid) at all concentrations. GSE 2 showed antioxidant activity at 25 μg/ml and above. Bagasse extract had considerably low-antioxidant activity. GBE showed 105 and 210 mg/g antioxidant activity at only 50 and 100 μg/ml, respectively. The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of a green Mo(V) complex with a maximal absorption at 695 nm (Prieto et al., 1999). The antioxidants break the free radical chain by donating a hydrogen atom (Gordon, 1990). According to Jayaprakasha et al. (2003), the antioxidant activity of different extracts may depend on the presence of polyphenols which may act as reductons. They also reported that the grape seed extracts obtained with the different solvents exhibited various degrees of antioxidant capacity.

The results of the scavenging ability of the grape extracts on hydrogen peroxide are shown in Fig. 3. Hydrogen peroxide scavenging ability of the grape extracts was dependent on the extract concentration and it increased when the extract concentration increased from 10 μg/ml to 50 μg/ml. The percentage of the hydrogen peroxide scavenging activity was 77.8% for GSE 1, 72.9% for GSE 2 and 67.3% for GBE. On the other hand, at the same dose, BHA and BHT showed 70.4% and 68.5% hydrogen peroxide scavenging activity, respectively. The ability of scavenging hydrogen peroxide of the extracts and standards at 50 μg/ml decreased in order of GSE 1 > GSE 2 > BHA > BHT > GBE. According to the results, both grape seed extracts had stronger hydrogen peroxide scavenging activity than the BHA, BHT and GBE.

The measurement of hydrogen peroxide scavenging activity can be one of the useful methods determining the ability of antioxidants to decrease the level of prooxidants such as hydrogen peroxide (Pazdzioch-Czochra & Widenska, 2002). Although hydrogen peroxide itself is not very reactive, it together with superoxide radical anion can damage many cellular components (Kaur & Perkins, 1991).

Peroxide value is a widely used measure of the primary lipid oxidation indicating the amount of peroxides formed in fats and oils during oxidation. Antioxidant activity of the grape extracts was compared with those of BHA, BHT and control samples. The results given in Fig. 4 showed that all extracts reduced the oxidation rate of poppy oil at 70 °C in terms of formation peroxides. After eight weeks all extracts showed antioxidant effect in varying degrees on poppy oil compared with the control. The peroxide value of control sample increased from 2.5 meq/kg to 842.3 meq/kg during this time. In terms of retarding the formation of oxidation products the effectiveness of the extracts at 0.2% concentration and standards at 0.02% concentration can be put into the following order: GSE1 > GSE 2 > BHT > GBE > BHA with the values of 146.4, 175.2, 244.6, 263.5, 463.4 and 842.3 meq/kg, respectively. The antioxidant activities of GSE 1 and GSE 2 were considerably higher than those of BHA and BHT. On the

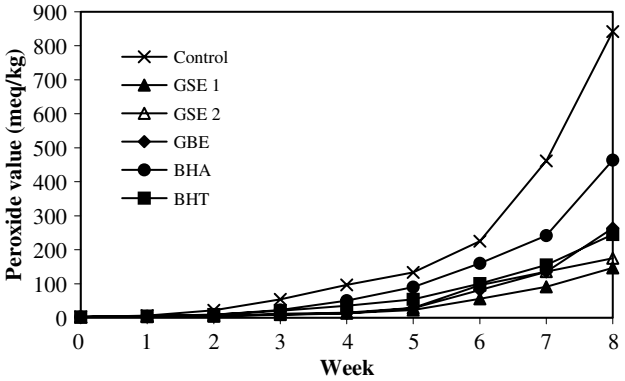

Fig. 4. Peroxide values of grape extracts (0.2%) and standards (0.02%) during storage at 70 °C for up to eight weeks in the dark.

other hand, GBE was more effective than BHA but less than BHT.

As a conclusion, the grape seed extracts showed strong antioxidant activity, by measuring their capacity to scavenge the DPPH and the hydrogen peroxide and to reduce Mo(VI) to Mo(V) and to decrease in the rate of peroxide formation, when compared to bagasse extract and standards.

In this study, it was determined that the grape seed extracts contained a higher amount of total phenolic content than the bagasse extract. As the grape seed extracts showed high-antioxidant activity also, it may be directly correlated to the high-phenolic contents of the seed extracts. In general, the antiradical and antioxidant activities of the plant extracts are ascribed to the phenolic contents (Lu & Foo, 2001; Miliauskas et al., 2004; Murthy et al., 2002; Özkan, Sağdıç, Göktürk Baydar, & Kurumahmutoğlu, 2004; Revilla & Ryan, 2000). Besides antiradical and antioxidant activities, anticancer activity of grape extracts was reported by Yi, Fischer, and Akoh (2005). They were found that the muscadinia grape skins had rich in phenolic acids and flavonoids, and skin anthocyanin fractions more than 90% pure showed more cancer inhibitory activity than phenolic acids. Bakkalbaşı, Yemiş, Aslanova, and Artık (2005) also determined that Narince seeds have a large scale of phenolics including gallic acid, catechin, epicatechin, total monomeric flavan-3-ol, total procyanidin and total flavan-3-ol as the values of 47, 279, 351, 629, 8111 and 8740 mg/100 g seed, respectively.

The results of the present study indicate that grape extracts can be used as easily accessible source of natural antioxidants. On the other hand, it will be extremely useful to utilize the wastes of wine-making process as alternative natural antioxidants to the synthetic antioxidants used in food industry to prolong the shelf life of food.

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