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DETERMINATION OF THE PHENOLIC COMPOUNDS, ANTIOXIDANT AND ANTIRADICAL ACTIVITIES OF SENIRKENT KARASI GRAPE CULTIVAR'S SKIN AND SEEDS

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Abstract

Purpose of this study was to determine total phenolic content, phenolic composition, antioxidant and antiradical activities of 'Senirkent Karası' grape cultivar's skin and seeds. While total phenolic contents of grape skin and seeds were determined by Folin-Ciocalteu method spectrophotometrically expressing the results in terms of gallic acid (GAE), phenolic composition was analyzed by HPLC (High Performance Liquid Chromatograph). Antioxidant activities of the grape skin and seeds were evaluated by reducing powers whereas antiradical activities were examined using DPPH (1-diphenyl-2- picrylhydrazyl). Results showed that total phenolic contents of seeds and skin were 52.32 and 1.89 mg g^{-1} GAE g^{-1} DM, respectively. Antiradical activities of seed and skin extracts (100 ppm) were 95.90 and 16.22 %, respectively. Reducing powers of seeds were 1.64 at 250 ppm, and were 2.39 at 1000 ppm whereas antioxidant activities) of skins were 0.08 at 250 ppm; 0.31 at 1000 ppm. Results showed that skin had higher amount of phenolic compounds than seeds and gallic acid, catechin, cafeic acid, syringic acid, resveratrol, quarcetin, kaempherol, p-cumaric acid were present in skin whereas only gallic acid, catechin, epicatechin were present in seeds. Seeds had the highest values of epicatechin (746.94 µg g^{-1}) while skins had the highest values of syringic acid (17.01µg g^{-1}) and gallic acid (5.29 µg g^{-1}).

Key words: *phenolics, antioxidant activity, antiradical activity, 'Senirkent Karası'.*

INTRODUCTION

There is an increasing interest on grape, grape products and other parts of grapevine due to rich chemical compounds they have. Increasing interest on natural antioxidants resulted in an increase in number of research on improvement and evaluation of natural products that are rich in phenolic compounds. Phenolic compounds that have very high antioxidant and antiradical properties are substances that have direct effect on quality, that give resistance ability to diseases and have pharmacologic features (Macheix et al., 1990; Clausen et al., 1992; Ayed et al., 1999; Yi et al., 2006). In addition, phenolic compounds reduce risk of cancer and heart diseases and lead to low density lipoprotein (LDL) due to their high antioxidant properties. There are studies showing that grape skin and seeds have a variety of polyphenol contents, high antioxidant property and contain flavonoids (catechin, epicatechin, procyanidins, anthocyanins), phenolic acids (gallic acid, ellagic acid) and stilbenes (resveratrol and piceids) (Jayaparakasha et al., 2003; Negro et al., 2003; Yılmaz and Toledo, 2006). However different parts of grape have different content of these above mentioned compounds.

'Senirkent Karasi' grape cultivar is a local grape grown in Isparta province that is mainly used as wine, table and dried consumption grape.

Thus, the purpose of this study was to determine the total phenolic content, phenolic composition, antioxidant and antiradical activities of 'Senirkent Karasi' grape cultivar's skin and seeds.

MATERIALS AND METHODS

Materials

In the study skin and seeds of 'Senirkent Karasi', a commonly grown cultivar in Isparta, was used. Fresh grapes were obtained from Isparta Directorate of Provincial Food Agriculture and Livestock, dried in the shade and later seeds and skin were separated to be analyzed. There were three replications for each analysis.

Phenolic extraction

Grape seeds and skins were manually separated from whole berries, seeds were dried at room temperature and then were crushed in a grinder for two min. 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 and also powdered skin were extracted in a *Soxhlet* apparatus for 8 h with 200 ml of acetone: water: acetic acid (90:9.5:0.5) at 60°C as described by Jayaprakasha et al (2003). The extracts were concentrated by rotary evaporator at 70°C to get crude extracts and stored in a desiccator.

Determination of total phenolic content

Total phenolic contents of the grape seed and skin extracts were determined spectrophotometrically using a PG Instruments T70 Plus Dual Beam Spectrophotometer (Arlington, MA, USA) according to the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965), calibrating against gallic acid standards and expressing the results as mg gallic acid equivalents (GAE g^{-1}) extract for seed and skin extracts. Data presented are average of three measurements.

HPLC determination of phenolic compounds

Chromatographic analyses were carried out on a Shimadzu model HPLC system (Shimadzu Corp., Kyoto, Japan). Separation of phenolics was performed by the modified method of Caponio et al. (1999). Reversed phase (RP)-HPLC analysis was done using a SCL-10Avp system controller, a SIL- 10AD vp autosampler, a LC-10AD vp pump, a DGU-14a degasser, a CTO-10 A vp column heater, and a Diode Array Detector with wavelengths set at 278 nm. The 250 x 4.6 mm i.d. 5 µm column used was filled with Agilent Eclipse XDB-C18 (Wallborn, Germany). The flow rate was 0.8 ml min^{-1}, the injection volume was 20 µl, and the column temperature was set at 30°C. For gradient elution, mobile phase A contained 3% acetic acid in water; solvent B contained methanol. The following gradient was used: 0-3 min, from 100% A to 95% A; 3-20 min, from 95% A to 80% A; 20-30 min, from 80% A to 75% A; 30-40 min, from 75% A to 70% A; 40-50 min 70% A to 60% A; 50-55 min, 60% A to 50% AB; 55-65 min, 50% A to 0% A. The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. The grape samples, standard solutions and mobile phases were filtered by a 0.45 µm pore size membrane filter (Millipore Co. Bedford, MA). The amount of phenolic compounds in the seed and skin extracts were calculated as mg 100 g^{-1} extract, separately, using external calibration curves obtained for each phenolic standard. Caffeic acid, (+)-catechin, chlorogenic acid, o-coumaric acid, p-coumaric acid, (-)-epicatechin, ferulic acid, gallic acid, kaempherol, trans-resveratrol, quercetin, syringic acid and vanillin acquired from Sigma (St. Louis, MO, USA) were used as standards and determined in the samples.

Determination of antiradical activity

The free radical scavenging activity of extracts were examined by comparing to those of known antioxidants such as BHT (butylated hydroxytoluene), BHA (Butylated hydroxyanisole) and trolox by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) from Sigma (St. Louis, MO, USA) using the method of Shimada et al. (1992). Briefly, a 1.0 ml solution of the samples (seed and skin extracts) and standards at 100 µg ml in methanol was mixed with 1.0 ml of methanolic solution of DPPH (0.2 mM). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm against methanol as the blank in a PG Instruments T70 Plus Dual Beam Spectrophotometer (Arlington, MA, USA). The addition of the samples 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 samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity (Baumann et al., 1979). Antioxidants break the free radical chain of oxidation and donate hydrogen from the phenolic hydroxyl groups. Therefore, the resulting stable endproduct does not permit further oxidation of the lipid (Sherwin, 1978). All determinations were done in triplicate and the percent of DPPH decolouration of the samples were calculated according to the formula:

Antiradical activity (%) = 100x[(absorbance of control-absorbance of sample)/absorbance.

Determination of reducing power

The reducing power of samples were determined by Oyaizu method (1986). Absorbance of supernatant was measured at 700 nm and compared to three standards, BHA, BHT and trolox; any increase in absorbance is synonymous of an increase in reducing power.

RESULTS AND DISCUSSIONS

Total phenolic compound content, antiradical and antioxidant activity of seed and skin are presented in Table 1.

As it is observed in Table 1, the yields (dry weight) of grape seed and skin had 12.60% and 9.64 %, respectively.

Table 1. Yield, total phenolic compound content, antiradical and antioxidant activity of seed and skin of 'Senirkent Karasi' grape cultivar

Sample	Yield (%)	Total phenolic content (mg/g GAE)	DPPH (100 ppm extract (%)	Reducing power (μgl^{-1}) (Absorbance)	
				250 ppm	1000 ppm
Seed	12.60±0.63	52.32±3.25	95.90±1.03	1.64±0.18	2.39±0.20
Skin	9.64±0.68	1.89±0.29	16.22±0.80	0.08±0.00	0.31±0.01

Total phenolic contents of the samples were estimated with Folin-Ciocalteu colorimetric method. When total phenolic contents of seeds extracts were calculated as mg GAE g^{-1} (Table 1) it is found that seeds had higher total phenolic compound content than skins. Seeds and skin had total phenolic compound content of 52.32±3.25 and 1.89±0.29 mg/g, respectively, in terms of gallic acid. Results are in agreement with those found by (Negro et al., 2003; Yılmaz and Toledo, 2004; Iacopini et al., 2008). These researcher also found that seeds had higher total phenolic compound content than skin.

HPLC method for analyzing phenolics in the samples has some advantages, such as easy and time consuming procedure for preparation of the samples, possibilities of quantification of a greater amount of diverse phenolics, the precision, accuracy and detection limits obtained for the phenolics quantified by this method enabling its application to grape (Gomez Alonso et al., 2007). The amounts and variations of phenolic compounds in the seed and skin extracts were determined by HPLC and presented in Table 2.

Table 2. Phenolic compounds of seeds and skin of 'Senirkent Karasi' grape cultivar

Phenolic compound	Skin, µg. g^{-1}	Seeds, µg. g^{-1}
Gallic acid	5.29±0.10	144.76±0.45
(+)-Catechin	3.43±0.21	637.88±5.55
(-)-Epicatechin	nd	746.94±2.13
Caffeic acid	3.83±0.29	nd
Syringic acid	17.01±0.21	nd
p-coumaric acid	0.70±0.01	nd
Trans-Resveratrol	2.47±0.02	nd
Ouarcetin	2.49±0.20	nd
Kaemferol	0.50±0.02	nd

It is found that skin had higher number of phenolic compounds than seeds. In skin samples 8 compounds such as gallic acid, catechin, caffeic acid, syringic acid, p-coumaric acid, resveratrol, quarcetin and kaempferol were detected whereas in seed samples only 3 compounds such as gallic acid, epicatechin and catechin were detected. Gallic acid amount in seeds and skin were determined as 144.76±0.45 µg g^{-1} and 5.29 ±0.10 µg g^{-1}, respectively. In

the same manner catechin amount in seeds and skin were determined as 637.88 ±5.55 μg g^{-1} and 3.43 ±0.21 μg g^{-1}, respectively. As regards to the presence of catechin in skin and seeds, it is commonly known that flavan-3-ols are located in both grape skin and seeds; however, skin contains much lower concentrations of flavan-3-ols than seeds (Revilla and Ryan, 2000).

In addition, another flavonoid, epicatechin, amounted in seeds 746.94±2.13 μg g^{-1} and it was not detected in skin. The results agree with the studies of Cheynier (1998), Rodriquez Montealegre et al. (2006) and Baydar et al. (2011), who also found that grape seeds had higher flavanol contents than skins. Another study also found that there was presence of epicatechin in seeds, whereas there was no epicatechin in skin (Souquet et al., 2000).

Trans-Resveratrol, a phytoalexin that belongs to the group of compounds known as stilbenes, is known to occur in grapes and consequently in grape products and in wine. *Trans*-resveratrol was found in 2.47 μg g^{-1} in the skin extracts. Baydar et al. (2011) also found 1.82 and 4.02 mg 100 g^{-1} of *trans*-resveratrol in grape skin extract. Iacopini et al. (2008) explained this result as the consequence of the fact that grapes produce stilbenes in response to mold infections and physiological stresses. If these stresses are not present, the levels of stilbenes in grapes remain low.

Radical scavenging activities of grape extracts, and standards were tested by the DPPH method. When radical scavenging activities of seed and skin is examined, it is observed that seeds had 95.90% antiradical activity whereas skin had 16.22% antiradical activity. The radical scavenging activities of the seed extracts were considerably higher than those of skin extracts. Grape seed extracts almost completely inhibited DPPH absorbtion. Otherwise skin extract contained remarkably lower amounts of radical scavenging compounds. Some researcher reported that there was a correlation between DPPH activity and total phenolic compound content of seed (Guendez et al., 2005; Hua et al., 2008). In this research it is also found that seeds had higher total phenolic compound content than skins and seeds had higher DPPH activity than skin.

When the reducing powers of seeds and skin was examined it was found that seeds had 1.64 μg l^{-1} and 2.39 μg l^{-1}values at 250 and 1000 ppm, respectively, whereas skin had 0.08 μg l^{-1} and 0.31 μg l^{-1} reducing power ability values at 250 and 1000 ppm, respectively. Higher absorbance values correspond to higher reducing power thus it is found that seeds had higher reducing power than skin. Hua et al. (2008) reported that in seeds of grapes there was a correlation between reducing power. In this research it is also found that seeds had higher total phenolic compound content than skins and seeds had higher reducing power than skin.

CONCLUSIONS

In this research we determined phenolic compounds, antiradical and antioxidant activity in seeds and skin of 'Senirkent Karasi' grape cultivar which is commonly produced and consumed in Isparta province.

The results obtained in this study showed that large differences were found grape seed and skin in relation to the phenolics composition. Senirkent Karası grape's seeds, and skins contained different phenolics with different levels and these variations affected the antioxidant capacity of the samples. Total phenolic contents, reducing powers of grape seed extracts are higher than those of grape skin extracts.

The result of study is important because grape seeds and skin are a good source of phenolic compounds that have positive effect on health, and they are rich in natural antioxidants. Thus, determining these compounds in a local cultivar is important in terms of health issue.

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REFERENCES

Ayed N., Yu H., Lacroix M., 1999. Improvement of anthocyanin yield and shelf-life extension of grape pomace by gamma irradiation. Food Research International, 539-43.

Baumann J, Wurm, G., Bruchhausen, F., 1979. Prostaglandin synthetase inhibiting O2-radical scavenging properties of some flavonoids and related

phenolic compounds. Naunyn-Schmiedebergs Archives of Pharmacology, 30: 27-32.

Caponio F, Alloggio V., Gomes T., 1999. Phenolic compounds of virgin olive oil:Influence of paste preparation techniques. Food Chemistry 64: 203-209.

Cheynier V., Labarbe B., Moutounet M., 2001. Estimation of proantho proanthocyanidin chain length. Methods in Enzymology, 335, 82-87.

Claussen T.P., Reichardt P.B., Bryant J.P., Provenza F., 1992. Condensed tannins in plant defense, a perspective on classical theorses, Plant Polyhends, Ed. By R.W. Mennengway and P.E. Laks Plenum, New York.

Gomez-Alonso S., Garcia R.E., Hermosin Gutierrez I., 2007. HPLC analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence. Journal of Food Composition and Analysis, 20: 618-626.

Guendez R., Kallithraka S., Makris D.P., Kefalas P., 2005. Determination of low molecular weight polyphenolic constituents in grape (*Vitis vinifera* sp.) seeds extracts: Correlation with antiradical activity. Food Chemistry, 89: 1-9.

Hua L., Xıaoyu W., Peıhong L., Yong L., Hua W., 2008. Comparative study of antioxidant activity of grape (*Vitis vinifera*) seed powder assessed by different methods. Journal of Food and Drug Analysis, 16 (6): 67-73.

Iacopini P, Baldi M., Storchi P., Sebastiani L., 2008. Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, *in vitro* antioxidant activity and interactions. Journal of Food Composition and Analysis 21: 589-598.

Jayaparakasha G.K., Selvi T., Sakariah K.K., 2003. Antibacterial and antioxidant activities of Grape (*Vitis vinifera*) Seed Extracts. Food Research International, 36, 117-122.

Macheix J.J., Fleuriet A., Billot J., 1990. Fruit Phenolics, CRC Press. Boca Raton, FL.

Negro C., Tommasi A., Miceli A., 2003. Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresour. Technology, 87, 41-44.

Oyaizu M., 1986. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition, 44, 307–315.

Revilla E., Ryan J., 2000. Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by HPLCphotodiode array detection without sample preparation. Journal of Chromatography A 881: 461- 469.

Rodriguez Montealegre R., Romero Peces R., Chacon Vozmediano J.L., Martinez Gascuena J., Garcia Romero E., 2006. Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. Journal of Food Composition and Analysis. 19, 687-693.

Shimada K., Fujikawa K., Yahara K., Nakamura T., 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry, 40, 945-948.

Singleton V.L., Rossi J.R., 1965. Colorimetry of total phenolics with phosphomolibdic - phosphothungstic acid. American Journal of Enology and Viticulture, 16, 144-158.

Souquet J.M., Labarbe B., Le Guerneve C., Cheyneir V., Moutounet M., 2000. Phenolics composition of grape stems. Journal of Agricultural and Food Chemistry, 48, 1076-1080.

Yilmaz Y., Toledo R.T., 2004. Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. Journal of Agricultural and Food Chemistry, 52: 255-260.

Yi W., Akoh C., Fischer J., Krewer G., 2006. Effects of phenolic compounds in blueberries and muscadine grapes on hepg2 cell viability and apoptosis. Food Research International, 628-638.


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