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Antioxidant Activity of Grape Seeds Obtained from Molasses (Pekmez) and Winery Production

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ABSTRACT

Grape seeds are good sources of phytochemicals and are suitable raw materials for the production of antioxidative dietary supplements. In this present study, the effect of waste origin (byproducts of winery and grape pekmez production) on the antioxidant activities, total phenol and flavonoid contents of grape seeds were determined. Antioxidant activities of grape seeds from pekmez production determined by FRAP, DPPH and ABTS assays were about 36.2, 40.8 and 26.8 µmol Trolox equivalent per gram dry matter, respectively. These values were twice higher than those from winery byproducts. Flavonoid content of grape seeds from pekmez production was 49.2 mg catechin equivalent per gram dry matter, and this was twice higher than that of winery waste. This study indicated that in terms of phenolic content and antioxidant activity, byproduct grape seeds of pekmez production are more valuable than those of winery waste.

Key Words: Antioxidant activity, Grape seed, ABTS, DPPH, FRAP, Calkarasi

Pekmez ve Şarapçılık Atıklarından Elde Edilen Üzüm Çekirdeklerinin Antioksidan Aktivitesi

ÖZET

Üzüm çekirdekleri fitokimyasallar açısından zengin bir kaynak olup, antioksidan gıda takviyesi üretimi için uygun bir hammaddedir. Bu çalışmada şarapçılık ve pekmez üretimi yan ürünleri olmak üzere atık kaynağının üzüm çekirdeklerinin antioksidan aktivite, toplam fenolik madde içeriği ve flavonoid içeriği üzerine etkisi belirlenmiştir. FRAP, DPPH ve ABTS yöntemleriyle belirlenen ve pekmez atıkları olan üzüm çekirdeklerinin antioksidan aktiviteleri sırasıyla 36.2, 40.8 ve 26.8 µmol Trolox eşdeğeri/g kuru madde olmuştur. Bu değerler şarapçılık atıklarından elde edilen değerlerden iki kat daha büyüktür. Pekmez atıklarından elde edilen çekirdeklerin flavonoid içeriği ise 49.2mg kateşin eşdeğeri/g kuru madde olup, şarapçılık atıklarından elde edilenlerden iki kat daha yüksektir. Bu çalışma sonuçları, pekmez üretiminde elde edilen atık üzüm çekirdeklerinin toplam fenolik madde içeriği ve antioksidan aktivite açısından şarapçılık atıklarından elde edilenlerden daha değerli olduğunu göstermektedir.

Anahtar Kelimeler: Antioksidan aktivite, Üzüm çekirdeği, ABTS, DPPH, FRAP, Çalkarası

INTRODUCTION

Grape (*Vitis vinifera*) is one of the world's largest fruit crops with an annual production of more than 60 million metric tons, and 80% of this production is used in wine production [1]. Some 10 million tons of grape pomace are produced as byproducts of this industrial wine production, and on a dry matter basis 38–52% of the pomace is composed of grape seeds [2]. Grapes are consumed as fresh or processed into raisins, juices wines and other products. Muslim countries mostly consume grapes as table grapes, grape juice, grape pekmez or raisins, whereas in European Mediterranean and North America, the main use of grapes is in wine production. Grape seeds are usually byproducts of processed grape products such as grape juice, grape pekmez and wine production. Monomeric phenolic compounds, such as (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-O-gallate and dimeric, trimeric and tetrameric procyanidins are dominant phenolic substances in grape seeds, and these compounds have antimutagenic and antiviral activities [3].

Calkarasi is a red variety of *V.vinifera*, endemic to the Western part of Turkey. This red variety is widely used for both red wine and grape pekmez production in Turkey. Differences in processing steps of wine and grape pekmez have a great influence on the composition and value of byproduct grape seeds and skins. Wine is produced by crushing grapes first to obtain must. White wines are made from white grapes, and berries are pressed immediately after crushing in order to separate the juice from grape seeds and skins. However, in red wine making, berries are crushed, and must is obtained. This must is pumped to a fermentor, and during maceration some of the health beneficial constituents of grape seeds and skins are transferred from must to wine besides color, aroma and taste compounds. When maceration is over, must is pressed to remove grape seeds and skins from the fermented juice. Grape skins and seeds are major byproducts of the winemaking industry. In the production of grape pekmez, seeds and skins of grapes are removed immediately after crushing the berries in order to obtain relatively clear juice. Grape juice is boiled with a calcareous substance to neutralize acidity, and then the clarified must is concentrated up to 70-80% soluble solids content [4].

In this present study, the antioxidant activities of grape seeds obtained from two different origins (byproducts of winery and grape pekmez production) were determined and the total phenol and flavonoid contents of grape seeds were compared.

MATERIALS and METHODS

Materials

Chemicals

All the chemicals were of analytical grade. Solvents used in antioxidant assays were of HPLC grade. Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Fluka (Switzerland) while Iron(III) chloride hexahydrate ($FeCl_3.6H_2O$), sodium carbonate were from Riedel-de Haen (Germany). Folin-Ciocalteu reagent and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS) were purchased from Merck (Germany). Trolox^{TM} (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and (+) catechin were obtained from Sigma (St. Louis, MO, USA).

Plant Material

Grape seed samples were of *Vitis vinifera* L. cv. Çalkarası berries (red variety) grown during the 2008 harvest season on vineyards located in Bekilli, Denizli, Turkey. Winery byproducts were obtained from Küp Winery Ltd. (Denizli) while byproducts of grape pekmez production were from Hancalar Pekmez Plant (Denizli). Samples were obtained from four different production lots, representing four replicates for each origin. After grape pomaces were dried in a tent type of solar drier, skin, stems and twigs were removed manually. Dried grape seeds were stored at 4°C until use.

Methods

Production of Grape Seed Extracts

A coffee grinder (Bosch, MKM 6000, Type: KM 13) was used for a minute to grind grape seeds into a powder form. Dry matter contents of grape seed powder samples were determined gravimetrically according to AOAC [5]. Grape seed extracts were prepared by mixing the powders with aqueous methanol (70%, v/v) at a ratio of 1:10 (w/v). The mixture was sonicated for 15 min and shaken for 30 min at room temperature followed by centrifugation at 4°C for 20 min at 26,000*g*. Clear supernatants were collected with glass Pasteur pipettes and poured into amber vials. This extraction procedure was repeated twice for the residue at the centrifuge bottle. Pooled supernatants were kept at -24°C until analyses.

Total Phenol Contents

Total phenol contents of grape seed extracts were determined by the Folin-Ciocalteu method [6]. Gallic acid was used as a standard. UV-Vis spectrophotometer with 8 cells (T80 Model, PG Instruments, England) was used to determine total phenol contents of extracts in terms of gallic acid equivalents (GAE).

Flavonoid Content

Flavonoid contents of grape seed extracts were determined spectrophotometrically using a procedure described by Zhishen et al. [7]. Catechin was used as a standard to determine flavonoid contents of grape seed extracts. The linear standard curve between 20 and 100mg/L catechin was used to express the results in mg catechin equivalent (CE)/g dry matter.

Antioxidant Activity Assays

The FRAP, DPPH and ABTS assay procedures described by Thaipong et al. [8] were used to determine antioxidant activities of grape seeds extracts. For FRAP assay, absorbance of ferrous tripyridyltriazine complex was measured at 593nm with a spectrophotometer (T80 Model, PG Instruments, England). For DPPH assay, the absorbance readings of extracts were taken at 515nm wavelength. The linear standard curves used in both FRAP and DPPH assays were between 10 and 50µM Trolox^{TM}. The absorbance values were measured at

734nm wavelength for ABTS assay, and the linear standard curve was between 5 and 25µM Trolox™. Antioxidant activity of grape seed extracts were expressed in µmol TE/g dry matter.

Statistical Analysis

Data were analyzed using the Statistical Analysis System software (SAS Institute) [9]. Student's t test was used to determine significant differences between the means. PROC CORR was used to determine Pearson's correlation coefficients (R) among the parameters studied.

RESULTS and DISCUSSION

Since grape pekmez production does not include any mass transfer of phenolic substances from berries to juice, byproduct seeds and skins retain health beneficial constituents after processing. Therefore, the health beneficial quality of byproduct grape seeds and skins from red grape pekmez production is considered more valuable than winery byproducts (specifically byproducts of red wine making). Maceration conditions in red wine production have a significant effect on the composition of antioxidant substances in byproduct grape seeds. Wines for early consumption are produced with 3–5 days of macerations time, and this time is usually enough to produce wines with good coloration and low tannins; however, wines for long aging are usually macerated on the seeds and skins from 7 to as long as 21 days [10].

In this present study, moisture content of byproduct grape seeds from grape pekmez production was significantly higher than winery byproduct ($p<0.05$) (Table 1). Major constituent of grapes is water, which is essential in numerous chemical reactions during grape growth and processing into a variety of products. Glucose and fructose are the predominant sugars in grapes and concentrations of these sugars depend on variety and maturity of grapes. Enzymatic hydrolysis of sucrose into glucose and fructose occur during wine fermentation. Sugar hydrolysis during fermentation might have an influence on the lower moisture content of byproduct grape seeds from wine production in comparison to grape seeds from pekmez production.

Table 1. Chemical properties and antioxidant activities of byproduct grape seeds from winery or grape pekmez production (n=4)

Property	Origin of Grape Seed*	
	Winery	Grape Pekmez Production
Moisture (%)	6.11 ± 0.09^b	7.41 ± 0.69^a
Total Phenol Content (mg GAE/g dm)	27.92 ± 7.42^b	54.61 ± 6.35^a
Flavonoid Content (mg CE/g dm)	24.16 ± 11.85^b	49.20 ± 7.08^a
Antioxidant activity (µmol TE/g dm)		
FRAP	19.96 ± 6.84^b	36.23 ± 5.50^a
DPPH	19.30 ± 7.04^b	40.79 ± 3.16^a
ABTS	16.45 ± 2.70^b	26.76 ± 0.07^a

*Superscripts with different letters within a row show significant differences at $\alpha=0.05$.

Total Phenol and Flavonoid Contents of Grape Seeds

Grape seeds are rich in polyphenolic constituents, and total phenol contents of seeds are dependent on the variety of grapes [11]. Maturation level and variety of grape berries have a significant effect on the flavan-3-ol and procyanidin contents of grape seeds, and the former was reported to reduce while the latter increase during maturation of berries [12]. In this present study, total phenol and flavonoid contents of byproduct grape seeds from pekmez production were 54.61mg GAE and 49.20mg CE per gram dry matter, respectively. And these values were significantly and twice higher than byproduct grape seeds from wine production ($p<0.05$) (Table 1). In total phenol and flavonoid contents of grape seeds, higher relative standard deviations were found for winery byproducts than pekmez byproducts. Wine making includes a maceration step, and this step is dependent on processing conditions. Higher variation in the total phenol and flavonid contents of winery seeds is more likely to arise from the differences in enological practices.

Antioxidant Activity of Grape Seeds

Ferric reducing antioxidant power of the grape seeds from winery byproduct was about 20µmol TE/g dm, and this was significantly lower than the seeds of pekmez production ($p<0.05$) (Table 1). Maier et al. [2] reported that ferric reducing antioxidant power of grape seeds was dependent on the variety of grapes. Determining antioxidant activities of peel, pulp and seed fractions of 28 fruits, Guo et al. [13] found that red rose grape seeds had the highest FRAP value (55mmol $FeSO_4$ equivalent/100g wet weight) among the seed fractions. Catechin and epicatechin, monomeric constituents of grape seeds, were shown to exhibit ferric reducing antioxidant power [14]. In our study, grape seeds of Calkarasi variety obtained from two different origins also exhibited DPPH radical scavenging activity. DPPH radical scavenging activity of byproduct grape seeds from pekmez production was about 41µmol TE/g dm, and this was significantly higher than the seeds from winery ($p<0.05$) (Table 1). Seeds of red grapes such as Merlot, Cabernet, Okuzgozu, Bogazkere and Kalecik Karasi were shown to exhibit DPPH radical scavenging activity [11, 15-17]. ABTS radical scavenging activity of byproduct grape seeds from pekmez production was about 27µmol TE/g dm, and this was significantly higher than the seeds from winery ($p<0.05$) (Table 1). Grape seeds and their phenolic constituents such as gallic acid, quercetin, catechin and epicatechin were reported to exhibit scavenging activity of grape seeds against DPPH or ABTS radicals [2, 14, 18-20].

Enological practices such as pressure application, clarification and maceration may influence phenolic composition and antioxidant activity of wines. High pressure rates increase both phenolic composition [21] and antioxidant activity of white wines [22]. Similarly, longer maceration times are able to increase the phenolic composition [23] and antioxidant activity of red wines as determined by ORAC, ABTS and DPPH assays [22]. Incorporation of heating to maceration not only enhances antioxidant activity of red wines as estimated by the ABTS method but also yields higher amounts of flavanols, anthocyanins and stilbenes in red wines than with traditional fermentation [24]. Differences in enological practices might be responsible for higher variation in the radical scavenging activity of winery grape seeds.

Storage temperature and water activity can influence catechin and epicatechin contents and antioxidant activity of grape seeds obtained from a red wine vinification process [14]. During red wine vinification, partial mass transfer of catechins and procyanidins into wine occurs [25]. A significant portion of phenolic compounds still remains in the seeds of pomace, and these compounds are health beneficial [26, 27]. Therefore, byproduct grape seeds of wine vinification are considered a valuable raw material for the recovery of phenolic compounds [28], and the interest in studying antioxidant activity of byproduct grape seeds is increasing [29-31].

Correlations among Antioxidant Activities of Grape Seeds and Their Phenolic Contents

Phenolic compounds are primary antioxidants which can donate hydrogen or electron, and radical intermediates can be stabilized by these types of compounds [32]. In a study, FRAP values of grape seeds were reported to be highly correlated with total phenol content and ABTS radical scavenging activity values of grape seeds [31]. In the present study, we also found that total phenol content were highly correlated with antioxidant activity (FRAP, DPPH and ABTS) of grape seeds (Table 2). A similar trend was also observed between the flavonoid content and antioxidant activity of grape seeds (Table 2). Thaipong et al. [8] reported a positive correlation coefficient between the total phenolic content and antioxidant activity of methanolic extracts of guava fruit. The present study exhibited that the total phenol content and flavonoid content of grape seeds can be a good indicator for the antioxidant activity of grape seeds.

Table 2. Pearson's correlation coefficients (R) between antioxidant activities of grape seeds and either their total phenol contents or flavonoid contents (n=8).

Antioxidant activity	Total Phenol Content	Flavonoid Content
FRAP	0.983	0.982
DPPH	0.984	0.961
ABTS	0.849	0.823

CONCLUSION

In general, grape seeds from pekmez production indicated significantly higher antioxidant activity than those from winery ($p<0.05$) (Table 1). Radical scavenging activity of byproduct seeds from pekmez production was measured with three different assays, and this activity was twice higher than byproduct grape seeds from wine production. In all three assays, byproduct grape seeds from winery wastes had higher relative standard deviations than the seeds from pekmez production, which might be due to the simpler production line of grape pekmez than wine production. The results of the present study indicated that total phenol and flavonoid contents of grape seeds are good indicators for their antioxidant activities.

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