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Characterization of grape seed and pomace oil extracts

By, **Nilgün Göktürk Baydar[1] , Gülcan Özkan[2,*] and Emine Sema Çetin[1]**

[1] Süleyman Demirel University, Faculty of Agriculture, Department of Horticulture, 32260 Isparta-Türkiye
[2] Süleyman Demirel University, Faculty of Agriculture, Department of Food Engineering, 32260 Isparta -Türkiye
Fax: +90-246-2370437; E-mail address: gozkan@ziraat.sdu.edu.tr

RESUMEN

Caracterización de extractos de aceite de orujo y pepita de uva.

El objetivo de este estudio fue determinar los contenidos de nutrientes y antioxidantes de extractos de aceite de orujo y pepita de uva de los principales cultivares de uva de Turquía, Kalecik karas1, Narince, Hasandede y Emir. El material procedente del orujo y las semillas, seco y pulverizado, se extrajo con hexano. Los resultados mostraron que la concentración de aceite de las semillas osciló entre 12,35 y 16,00 % mientras que en el aceite de orujo la concentración varió entre 5,47 y 8,66%. Los aceites de orujo y pepita de uva eran ricos en ácido oleico y linoleico y su grado de instauración fue de un 85%. El α-tocoferol fue el tocoferol más abundante en los extractos. Aunque también se encontraron γ y δ-tocoferol a bajas concentraciones, no se detectó la presencia de β-tocoferol. Los extractos de aceite de orujo mostraron el contenido de tocoferol más alto comparado con las semillas en todos los cultivares. Los contenidos de fenoles totales fueron más altos en los extractos de orujo que en los de semillas, encontrándose el valor más alto (392,74 mg/kg) en el extracto de orujo del cultivar Narince. Los índices de refracción de los extractos de aceite de orujo variaron de 1,445 a 1,468 mientras que los de semilla estuvieron entre 1,460 y 1,466. En conclusión, lo sub-productos del vino incluyendo las semillas y el orujo pueden ser utilizados para conseguir antioxidantes naturales y para obtener aceite vegetal comestible.

PALABRAS-CLAVE: Ácidos grasos - Fenoles totales - Orujo - Pepita de uva - Tocoferoles.

SUMMARY

Characterization of grape seed and pomace oil extracts.

The objective of this study was to determine the nutrient and antioxidant contents of grape seed and pomace oil extracts from the main Turkish wine grape cultivars, Kalecik karas1, Narince, Hasandede and Emir. Dried and powdered seed and pomace materials were extracted with hexane. The results showed that the oil concentration of seeds ranged from 12.35 to 16.00% while in pomace the oil concentration varied from 5.47 to 8.66%. Grape seed and pomace oils were rich in oleic and linoleic acids and the degree of unsaturation in the oils was over 85%. α-tocopherol was the most abundant tocopherol in the oil extracts. Although γ and δ-tocopherols were found with low concentrations, β-tocopherol was not detected in the oil extracts. Oil extracts from pomace in all cultivars gave the highest tocopherol contents compared to the seeds. The contents of total phenolics were higher in pomace oil extracts than seed oil extracts. The highest total phenolic content (392.74 mg/kg) was found in the oil extract from Narince pomace compared to the other oil extracts. The refractive indexes of pomace oil extracts ranged from 1.445 to 1.468 while the refractive indexes of the seed oil extracts ranged from 1.460 and 1.466. In conclusion, wine by-products including the seeds and pomace can be utilized both to get natural antioxidants and to obtain edible vegetable oil.

KEY-WORDS: Fatty acids - Grape seeds - Pomace - Tocopherol - Total phenolics.

1. INTRODUCTION

An interest in natural antioxidants, especially of vegetal origin, has greatly increased in recent years. Natural antioxidants can protect the human body from free radicals that may lead to the aging process and cause some chronic diseases including cancer, cardiovascular diseases and cataract as well as retard lipid oxidative rancidity in foods (Kinsella *et al.*, 1993; Lai *et al.*, 2001). Natural antioxidants include tocopherols and phenolic compounds which may act to confer an effective defence system against free radical attack. Many researches supported the theory that free radicals cause oxidative damage and contribute to the development of many illnesses and the aging of organisms (Cutler, 1991; Ames *et al.*, 1993; Gey, 1993). Antioxidants have been found in cereals (Yu *et al.*, 2002), plant and seed oils (Yu *et al.*, 2005; Owen *et al.*, 2000; Gimeno *et al.*, 2002), vegetables (Cao *et al.*, 1996) and fruits (Kalt *et al.*, 1999; Wang *et al.*, 1996).

Tocopherols are one of the most powerful natural fat-soluble antioxidants. Tocopherols can act as antioxidants by two primary mechanisms, a chain-breaking electron donor mechanism, in which they donate their phenolic hydrogen atom mechanism, which includes singlet oxygen scavenging or quenching; this inhibits oxidations induced by electronically excited singlet oxygen (Kamal-Eldin and Appelqvist, 1996). Oil extracts with high tocopherol content can be used in applications where a high level of antioxidant

protection is needed (Haumann, 1990; Demurin *et al*., 1996). On the other hand, among the tocopherols present in foods, α-tocopherol shows the highest vitamin E activity, thus making it the most important for human health and biological activity (Guthrie and Kurowska, 2001).

Phenolic compounds are able to donate a hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation (Shadidi, 1997). Although the interest in phenolic compounds is related primarily to their antioxidant activity, they also show important biological activity *in vivo* and may be beneficial in combating diseases related to excessive oxygen radical formation exceeding the antioxidant defence capacity of the human body (Morello *et al*., 2004).

In addition to providing tocopherols and phenolics, grape seeds and pomace are also an important oil source. Grape seeds have 10 to 20% oil (Schuster, 1992) and the oil is especially rich in unsaturated fatty acids (Göktürk Baydar and Akkurt, 2001). Unsaturated fatty acids are very important for the stability of oils because of the chemical reactions in the double bonds. The rates of those oxidation reactions depend on the number of double bonds in the carbon chain. Poly-unsaturated fatty acids such as linoleic and linolenic are essential for the human body because they cannot be synthesized in the body. From this point of view, grape seeds and pomace oil very rich in linoleic acid may be a valuable source of dietary fat.

Wine by-products containing some valuable substances such as fatty acids, tocopherols and polyphenols with potential application in the food industry mostly go to waste. Pomace, consisting of seeds, skins and stems, is an important by-product of winemaking and the production of traditional foods such as molasses and vinegar. An estimated 13% by weight of the grapes processed by the wine industry ends up as by-products after pressing (Torres *et al*., 2002). The use of pomace in the food industry can create some opportunities to lower production costs and to create a new food source for human consumption.

The aim of this work was to determine the fatty acid composition, α, γ, δ and total tocopherol contents, total phenolic contents and refractive index of oil extracts obtained from the seeds and pomace of four wine grape cultivars in order to examine their nutrient and antioxidant contents.

2. MATERIALS AND METHODS

2.1. Materials

Seeds and pomace (seed, stem and skin) of four wine grape cultivars, Kalecik karas› (red), Narince (white), Hasandede (white) and Emir (white) were used as materials in the present study. Pomace of each cultivar was taken from the winery of Ankara University (Ankara, Türkiye) in 2004 and divided into two parts. Seeds were picked from one part for obtaining the seed samples and the second part was used directly as pomace samples. Before the analyses, all seeds and pomace samples were air-dried at room temperature in the dark for three months.

2.2. ANALYSIS OF OIL CONTENT

Dried seeds and pomace were crushed in a grinder for two min, but at 15 s intervals the process was stopped for 15 s to avoid heating the sample. Oil content was determined according to the AOCS method (AOAC, 1990). The powdered grape seeds and pomace (4 g) were extracted in a Soxhlet extractor (Büchi Universal Extraction System B-811, Germany) for 6 h with 150 mL of hexane at 60 °C.

2.3. ANALYSIS OF FATTY ACIDS

The fatty acid composition of the oil extracts was determined by gas chromatography (GC). Fatty acid composition was performed using a method as given by Marquard (1987).

The chromatographic separation was performed in a Perkin Elmer Auto System XL gas chromatograph equipped with a flame ionization detector (FID), and a fused silica capillary column (MN FFAP (50 m x 0.32 mm i.d.; film thickness 0.25 μm). It was operated under the following conditions: oven temperature program, 120 °C for 1 min. raised to 240 °C at a rate of 6 °C/min and than kept at 240 °C for 15 min; injector and detector temperatures, 250 and 260 °C, respectively; carrier gas, helium at flow rate of 15 cm/s; split ratio, 1/20 mL/min. Fatty acids were identified by comparing retention times with standard compounds. Five fatty acids were considered in this study. These were palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3), acids expressed as percentages of fatty acids.

2.4. ANALYSIS OF TOCOPHEROLS

In the tocopherol analyses, the HPLC method of Lampi *et al.* (1999) was modified. Tocopherols (α, β, γ and δ-tocopherol) were evaluated by high-performance liquid chromatography with direct injection of an oil extract in a mixture of heptane:tetrahydrofuran (THF) (95:5) solution. Detection and quantification was carried out with a SCL-10Avp System controller, SIL–10ADvp Autosampler, LC-10ADvp pump, CTO-10 Avp column heater and fluorescence detector with wavelengths set at 295 nm for excitation and 330 nm for emission. The 150cm x 4,6 mm i.d. column used was filled with Supelcosil Luna, 5μ (Supelco, Inc. Bellefonte, PA). The mobile phase consisted of heptane/THF (95/5) (v/v) at a flow rate of 1.2 mL/min and the injection volume 10 μL. The data

were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. Standard samples of α, β, γ and δ isomers of tocopherol (Sigma Chemical Co., St. Louis, Mo., USA) were dissolved in hexane and used for identification and quantification of peaks. The amount of tocopherols in the extracts was calculated as mg tocopherols in kg oil extract using external calibration curves (r = 0.999), which were obtained for each tocopherol standard.

2.5. Analysis of total phenolic contents

Total phenolic contents of the oil extracts were isolated from a solution of oil extract in hexane by triple-extraction with water:methanol (60:40 v/v). The concentration of total phenolic compounds was estimated spectrophotometrically at 765 nm according to the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). Results were expressed as mg gallic acid per kg of oil extract. Data presented are the average of three measurements.

2.6. Analysis of Refractive Index (nD 20°C)

Refractive indexes of grape seed and pomace oil extracts were determined by using of the 60/70 Abbe-refractometer (Belligham + Stanley Ltd., England).

3. RESULTS AND DISCUSSION

3.1. Oil content

The oil contents of grape seeds and pomace extracted from four different wine-grape cultivars are given in Table 1. The oil contents of grape seeds ranged from 12.35 (Kalecik karas›) to 16.00% (Hasandede). These results are in agreement with those obtained by Ohnishi *et al.* (1990), Schuster (1992) and Göktürk Baydar and Akkurt (2001). They reported that the oil content of seeds obtained from different grape cultivars ranged from 9.90 to 20.00%. Similarly, Beveridge *et al.* (2005) using two extraction methods found that grape seed oil yields ranged from 5.85 to 13.6% and 6.64 to 11.17% with supercritical carbon dioxide and petroleum ether extractions, respectively. On the other hand, the oil contents of pomace varied from 5.47 to 8.66%. Hasandede pomace had the highest oil content value (8.66%) and the lowest oil content value (5.47%) was detected in Narince.

3.2. Fatty acid contents

The fatty acid composition of the oils extracted from four grape cultivars is shown in Table 1. The major fatty acid in grape seed and pomace oil was linoleic acid. The fatty acid contents of the grape seed oils had the following range: 7.42 to 10.24% for palmitic, 2.95 to 4.68% for stearic, 16.15 to 21.63% for oleic, 63.33 to 71.37% for linoleic and 0.14 to 0.35% for linolenic acid. These results are similar to those described by Göktürk Baydar and Akkurt (2001), Beveridge *et al.* (2005) and Baron *et al.* (1988). In the pomace oils, the contents ranged from 8.60 to 10.63%, 3.58 to 4.59%, 16.07 to 22.57%, 61.16 to 69.97% and 0.47 to 0.63% for palmitic, stearic, oleic, linoleic and linolenic acids, respectively. The fatty acid composition of grape seed and pomace are similar to the oils of safflower, sunflower, soybean, maize, cotton seed, popy and tobacco, which belong to the linoleic type.

Grape seed oil was rather poor in linolenic acid. Low levels of linolenic acid are desired in edible oils, because high levels of this fatty acid can produce an unfavourable odour and taste in oil. Furthermore, since linolenic acid is oxidised simply due to having three double bonds on its hydrocarbon chain, the stability or shelf-life of oil rich in linolenic acid would be short (Hall *et al.*, 1981; Mayes, 1983). So, grape seed and pomace oils having low quantities of linolenic acid, can be an advantage in terms of human consumption and the shelf-life of the oil. The second abundant fatty acid in seed and pomace oil was oleic acid. Oleic acid, a monounsaturated fatty acid, also has great importance in terms of their nutritional implication and the effect on oxidative stability of oils (Aparicio *et al.*, 1999).

Table 1
Oil content and fatty acid composition of some grape seeds and pomace.

Oil source	Cultivars	Oil content (%)	Fatty acid composition (%)					Degree of unsaturation
			Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	
Seeds	Kalecik karas1	12.35±0.45	10.24±0.19	4.04±0.12	21.63±1.22	63.33±3.86	0.35±0.04	85.72±3.27
	Narince	14.20±0.93	7.42±0.32	2.95±0.09	17.70±0.94	71.37±4.32	0.14±0.01	89.63±3.64
	Emir	15.12±0.59	8.75±0.12	4.68±0.34	16.15±1.23	69.70±3.05	0.26±0.01	86.57±4.52
	Hasandede	16.00±0.69	8.88±0.87	4.08±0.31	19.78±0.65	66.20±4.36	0.30±0.02	87.04±5.12
Pomace	Kalecik karas1	6.46±0.32	10.63±1.35	4.15±0.22	22.57±1.32	61.16±3.67	0.63±0.03	85.22±3.46
	Narince	5.47±0.06	9.29±0.76	3.58±0.16	18.89±0.46	66.76±2.38	0.53±0.02	86.83±4.93
	Emir	6.36±0.28	8.60±0.86	4.59±0.22	16.07±0.54	69.97±3.29	0.47±0.02	86.81±5.63
	Hasandede	8.66±0.29	9.09±0.93	4.08±0.32	20.15±1.56	65.64±3.19	0.55±0.01	86.83±2.64

The degree of unsaturation in grape seed and pomace oil was over 85%, coming from unsaturated fatty acids. High levels of unsaturation play an important role in lowering high blood cholesterol and also in the treatment of atherosclerosis (Axtell, 1981).

3.3. Tocopherol contents

α, γ, δ and total tocopherol contents of grape seed and pomace oil extract isolated from grape cultivars are presented in Table 2. While β-tocopherol was not detected in the oil extracts of seeds and pomace, α-tocopherol was the most abundant tocopherol in the oil extracts, as previously reported by Gliszczynska-Swiglo and Sikorska (2004). γ and δ-tocopherols followed the α-tocopherol with the lowest values. Pomace oil extract gave higher individual tocopherol contents than the seed oil extract. In the pomace oil extracts, contents ranged from 198.66 to 925.69, 27.53 to 111.42, 2.17 to13.59 mg/kg pomace oil extract for α, γ, δ and total tocopherols, respectively. On the other hand, values varied from 128.14 to 325.39 for α, 14.37 to 31.73 for γ, 0.62 to 1.63 mg/kg for δ-tocopherol in the seed oil extracts. Tocopherol contents varied not only from one oil extract source to another but also among cultivars. The seed and pomace oils of Kalecik karas› exhibited the highest α and total tocopherol values as compared to the other cultivars. Similarly Göktürk Baydar and Akkurt (2001) and Aguilera et al. (2005) reported that the tocopherol contents changed depending on the genotype. Published studies on the tocopherol contents of grapes are mostly focused on grape seed oil (Oomah *et al.*, 1998; Göktürk Baydar and Akkurt, 2001). Gliszczynska-Swiglo and Sikorska (2004) found α, γ+ β and δ-tocopherols to be 100.55 mg/kg, 17.14 mg/kg and 3.89 mg/kg, respectively.

3.4. Total phenolic contents

Because phenolic compounds may contribute to overall antioxidant activities, other natural antioxidants that can be found in grape seed and pomace oil extracts are phenolics. These are important antioxidants that protect the oil against autoxidation, at the cellular level, against the oxygen level (Aguilera *et al.*, 2005). The total phenolic content was determined using the Folin-Ciocalteu reagent. Total phenolic contents, estimated as gallic acid equivalents of oil extract, were found to be higher in pomace oil extracts rather than in seed oil extracts. Total phenolic contents in the seed oil extracts ranged from 100.64 mg/kg (Hasandede) to 238.47 mg/kg (Narince). Oil extract isolated from Narince pomace showed the highest total phenolic content value of 392.74 mg/kg oil extract and the lowest phenolic content value of 154.90 mg/kg oil extract was found in Emir pomace oil extract.

3.5. Refractive index

The refractive indexes of pomace oil extracts ranged from 1.445 to 1.468 while the values in the seed oil extracts varied from 1.460 to 1.466. The greatest refractive index was detected in the Hasandede pomace oil extract.

4. CONCLUSION

Oil extracts from the seed and pomace of different grape cultivars contain a large amount of unsaturated fatty acids, tocopherols and total phenolic compounds. The beneficial effects of grape seed and pomace oil extracts are due not only to their high degree of unsaturation, but also to their antioxidants such as tocopherols and phenolic compounds which may serve as dietary sources of natural antioxidants to prevent diseases and to promote human health. As a result, wine by-products, a large scale waste, can be utilized both to get natural antioxidants and to obtain edible vegetable oil.

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Table 2
Tocopherols, total phenolics and refractive indexes of grape seed and pomace oil extracts.

Oil source	Cultivars	Tocopherol contents (mg/kg oil extract)				Total phenolic content (mg/kg oil extract)	Refractive index
		α-tocopherol	γ-tocopherol	δ-tocopherol	Total tocopherol		
Seeds	Kalecik karas1	325.39±32.16	31.73±5.31	1.63±0.06	358.75±35.24	171.32±12.24	1.463
	Narince	175.80±12.75	39.31±1.12	0.70±0.06	215.81±13.82	238.47±14.07	1.462
	Emir	180.43±11.90	29.84±4.96	0.62±0.08	210.89±12.50	133.69±8.16	1.466
	Hasandede	128.14±8.72	14.37±3.07	1.54±0.09	143.05±10.06	100.64±7.62	1.460
Pomace	Kalecik karas1	925.69±32.96	111.42±6.51	13.59±2.31	1050.7±34.86	213.02±11.00	1.445
	Narince	619.71±16.52	76.48±11.67	14.32±1.81	710.51±17.42	392.74±21.56	1.459
	Emir	400.33±28.34	81.31±9.73	4.85±0.56	486.49±31.45	167.43±9.80	1.460
	Hasandede	198.66±10.86	27.53±2.36	2.17±0.46	228.36±12.16	154.90±8.48	1.468

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