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Valorisation of seeds from different grape varieties for protein, mineral, bioactive compounds content, and oil quality

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RESEARCH ARTICLE

Abstract

This study aimed to investigate the quality characteristics and bioactive properties of grape seed oil obtained from five different grape varieties (Öküzgözü-Elazığ, Syrah-Çanakkale, Cabernet Franc-Çanakkale, Pinot-Noir-Tekirdağ, and Merlot-Manisa) by cold press system. In addition, the antibacterial activity of grape seed oils was determined. The grape varieties significantly affected physicochemical properties, fatty acid and sterol composition of grape seed oils ($P<0.05$). The protein and oil, total flavonoid, phenolic, and anthocyanin contents of grape seeds changed between 7.44-13.66 and 6.93-8.80%, were 254-1,436.67, 4,397.93-5,804.29, and 0.31-7.89 mg/g, respectively. The acidity, peroxide number and tocopherol amount of grape seed oils obtained by cold press method were determined as 0.67-2.74%, 10.45-22.03 meqO_2/kg, and 2.04-11.595 mg /100 g oil, respectively. The most abundant fatty acids were palmitic, stearic, oleic and linoleic acids, and these values were 9.98-14.98, 3.61-6.97, 15.31-28.94 and 48.78-69.13% respectively. β-sitosterol exhibited major sterol for all varieties and ranged, 61.6 and 69.54%. All grape variety oils gradually showed antimicrobial activity at different ratios against *Staphylococcus aureus* (ATCC 2592), *Escherichia coli* (ATCC 25922), *Salmonella enterica* subsp. *enterica* serovar Enteritidis (ATCC 13076) and *Aspergillus parasiticus* DSM 5771. This study suggested that grape seed oil of selected varieties could be utilised as a good source of edible oil due to its antioxidant, essential fatty acids, sterol contents, and antibacterial properties.

Keywords: grape, variety, cold press, grape seed oil, physicochemical properties, fatty acid, tocopherol, sterols, phenolic compounds, antimicrobial activity, GC, HPLC

1. Introduction

Grapes is one of the most widely cultivated plants around the world. Most of the grapes are utilised for winemaking in both Turkey and the world. The rest is being consumed as fruit and used in nutraceutical or pharmaceutical industry. Grape seeds are a by-product of winemaking process, and they constitute a considerable proportion of the pomace (38-52%). During the wine production, thousands of tons of grape seed are revealed as a natural by-product, and its alternative usage should be improved (Pierron, 2017). Grape seed can be evaluated as a protein, oil, phenolic and antimicrobial source (Poveda *et al.*, 2018). Grape seed can be considered as a good source of alternative vegetable oil both regarding quantity and regarding the bioactive components (Lutterodt *et al.*, 2011).

Recently, grape seed oil has gained popularity as a culinary oil because of its positive health effect. Grape seed oil is composed of average 90% poly and monounsaturated fatty acids, which are responsible for its value as nutritive edible oil, particularly of linoleic acid (58-78%, 18:2n-6) followed by oleic acid (3-15%, 18:1n-9) and minor amounts of saturated fatty acids (10%) (Al Juhaimi and Ozcan, 2018; Beveridge *et al.*, 2005). Since it is a rich source of linoleic acid, it has promoted cardiovascular health by down-regulating low-density lipoprotein cholesterol.

Grape seed oil is being industrially produced by solvent extraction, however, in this study, it was obtained by cold pressing, as an alternative extraction process. The cold-pressing procedure involves neither heat nor chemical treatments, so it has better nutritive properties which consumer are looking for in natural and safe food products (Rombaut *et al.*, 2014). Moreover, cold press technique is simple and environment-friendly method since it does not require much energy (Al Juhaimi and Ozcan, 2018). Cold-pressed grape seed oil stands out as a suitable alternative to other common vegetable oils because it has a higher content of essential fatty acid, and many others bioactive compounds including phenolics, antioxidants, tocopherols, and sterols (Ben Mohamed *et al.*, 2016).

The aim of this study was to: (1) determine the physicochemical properties and bioactive properties of seeds of 5 different varieties of grape (Öküzgözü, Syrah, Cabernet Franc, Pinot-Noir and Merlot cultivated in Elazığ, Çanakkale, Tekirdağ, and Manisa respectively); and (2) fatty acid, sterol, and tocopherol composition and antibacterial effect of oil extracts obtained from those grape seeds.

2. Materials and methods

Materials

The maturated grapes (Öküzgözü-Elazığ, Syrah-Çanakkale, Cabernet Franc-Çanakkale, Pinot-Noir-Tekirdağ, and Merlot-Manisa) were provided from the commercial winery (Doluca Wine Factory, Çerkezköy, Tekirdağ, Turkey) in the harvest season. Grapes were separately crushed by hand, and their pulps were removed to obtain seeds. Before the analyses, all seeds samples were dried for in an oven for 24 h at 70 °C until reaching the 7-7.5% moisture content. The dried seeds were finally ground in a grinder, and the seed powders were kept in 4 °C before analysis.

Methods

Oil, protein and mineral content of grape seeds

Oil and protein content of the grape seed samples were determined according to the AOAC methods 920.39 and 920.87, respectively. The soxhlet extraction method was used for the determination of the total oil content of grape seed by using petroleum ether for four hours (AOAC, 1990). The extract was evaporated by a rotary evaporator (Buchi, rotavapor R-210, Flawil, Switzerland) equipped by a vacuum pump (Buchi, Vacuum Pump V-700, Flawil, Switzerland) at a 241 mPa. The lipid extract was collected in a flask. The extracted lipid was weighed to determine the oil content.

For mineral content analysis, 0.5 g of sample was digested in a closed microwave system by using 5 ml of 65% HNO_3 and 2 ml of 35% H_2O_2. Then 20 ml of ultra-deionised water was added, and the sample was examined by ICP AES (Varian-Vista, Mulgrave, Australia) for minerals (Skujins, 1992).

Extraction procedures

Methanol was used for the extraction of phenolic compounds from the grape seeds. Firstly, 50 ml of methanol was added to 5 g of powdered grape seeds and homogenised by Ultra-Turax (Daihan, HG-15D) at 10,000 rpm for 3 min. Then, the obtained solution was incubated at room temperature for 1 h in the shaking water bath (Memmert WB-22, Memmert GmbH, Büchenbach, Germany) for the extraction of the phenolic compounds. After the extraction process, the extracts were centrifuged (Hettich, Universal 320R, Tuttlingen, Germany) at 2,500 g for 10 min and filtered through Whatman No. 1 and 0.45-μm filter (Whatman International Ltd., Maidstone, UK).

Total phenolic contents

The total phenolic content of grape seed extracts was determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). Initially, 2.5 ml of 0.2 N Folin-Ciocalteu phenol reagent and 2 ml of 7.5% Na_2CO_3 were added to 0.5 ml of the methanolic extract. The obtained mixture was incubated for 30 min at room temperature in a dark place. At the end of the holding time, the absorbance was measured at 760 nm using a UV-vis spectrophotometer (Shimadzu, UV-1800). The total phenolic content was calculated as gallic acid equivalent. Estimations were carried out in triplicate and calculated from a calibration curve obtained with gallic acid. Total phenolic was expressed as gallic acid equivalents (mg GAE/g extract).

Total flavonoid contents

The total flavonoid contents of the seeds were estimated according to the method described by Dewanto *et al.* (2002). Methanol extracts were properly diluted with distilled water. Then 5% $NaNO_2$ solution was added to each test tube, and it was allowed to stand for five min. Then 10% $AlCl_3$ solution was added and, after six minutes, 1.0 M NaOH was added. The total volume was filled to 5 ml with water, and the test tubes were mixed. Solution absorbance was measured at 510 nm versus a blank. The calibration curve was prepared using catechol as standard.

Oil extraction by cold press

Grape seed oil was obtained by the cold-press extraction process. A cold press (Ekotok-1, Tokul Ltd. Co, Izmir, Turkey) was used for the extraction of grape seed oil. The press capacity was 6 kg seed production per hour and nozzles sizes were 5 mm. The temperature did not exceed 50±1 °C to preserve oil quality. After pressing, the oil was separated from solid impurities by filtering through the filter paper. Purified oil samples were kept in coloured bottles at 4 °C for further analyses.

Physicochemical quality analyses of oil samples

The free fatty acidity (FFA), acid number and the peroxide values (PV) of the oils were determined according to the methodology of the IUPAC 2.201 and 2.50, respectively (IUPAC, 1992).

Fatty acid composition

The oil samples were methylated using BF_3-methanol according to AOCS (1990). The fatty acid methyl ester was injected in gas chromatography (with a capillary column, HP-88, 100 m × 0.25 mm, film thickness: 0.20 mm) and analysed by gas chromatography (Agilent 6890N, Waldbronn, Germany) equipped with a flame-ionisation detector. The carrier gas was helium, with a flow rate of 0.5 ml/min. The temperatures of the injector and the detector were held at 250 and 280 °C, respectively. The initial oven temperature of 120 °C was maintained for 10 min, raised to 240 °C at a rate of 5 °C/min. The injection volume was 1 μl. The fatty acid methyl esters of grape seed oils were identified by comparing the retention time of the samples and appropriate fatty acids methyl esters standards. The percentage of individual fatty acid content, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and poly unsaturated fatty acid (PUFA) content are given.

Total tocopherol content

Total tocopherol content (mg of α-tocopherol per kg of oil) was determined by the HPLC method of AOAC (2000). The chromatographic separation was performed using the mobile phase consisted of ethyl acetate: acetic acid: hexane (1:1:98 v/v/v) at a flow of 1.5 ml/min. Detection was performed by the fluorescence detector at 290 nm (excitation) and 330 nm (emission) wavelengths. The number of tocopherols in the samples was calculated as μg tocopherols in ml oil extract using external calibration curves (0-10 μg ml r^2=0.999), which were obtained with the α-tocopherol standard.

Sterol composition

To determine sterol composition, 0.5 g of oil sample was weight into a test tube and saponified with 5.0 ml saturated methanolic KOH at 80 °C for one hour. It was then extracted with five ml of hexane three times; resulted solution was dried with anhydrous sodium sulphate. A 0.5 ml of dried hexane extract was silylated with solution of 0.1 ml bis (trimethylsilyl) trifluoroacetamide/trimethylchlorosilane (4:1 v/v). After pre-treatment, the sterol composition of the seed oils was determined using GC equipped with flame-ionisation detector. Separation of the sterols was performed using CP-SIL 24 CB (60 m × 0.32 mm × 1.00 μm), and the following method parameters were used for sterol composition analysis. Working conditions were as follows: carrier gas, helium; flow rate was 0.8 ml/min; injector temperature, 280 °C; detector temperature, 300 °C; oven temperature program, initial temperature was 50 °C for 2 min, increased at 60 °C/min to 245 °C, held for 1 min and then increased at 3 °C/min to 275 °C, held for 35 min (Kamm *et al.*, 2002).

DPPH radical scavenging activity

The antioxidant compounds of the oil samples were extracted with methanol (1:1 v/v) three times. The antioxidant activity values of methanolic extracts were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method according to (Singh *et al.*, 2002). Then, 0.1 ml extract was mixed with 2 ml methanolic DPPH solution, and the mixture was shaken and kept at room temperature for 30 min. The absorbance was measured at λmax 517 nm (UV-Mini 1240, Schimadzu, Kyoto, Japan) after 30 min in the dark at room temperature. The Trolox equivalents antioxidants capacity value is expressed as micromole Trolox equivalents per grams of cold press oil sample (TE μM/g).

Antibacterial activity

The disc diffusion method was used to detect the antibacterial activity of the extracts. Antimicrobial activity was screened against *Staphylococcus aureus* subsp. *aureus* (ATCC 2592), *Listeria monocytogenes* (ATCC 7644), *Salmonella enterica* subsp. *Enterica* serovar Enteritidis (ATCC 13076), *Escherichia coli* (ATCC 25922). For the disk diffusion assay, 0.1 ml of each bacterial suspensions were adjusted to 1×10^7 cfu/ml and uniformly spread on a solid growth medium in a Petri dish. The discs (5 mm in diameter) were individually impregnated with 10 μl of each oil and then placed onto the agar plates. Before incubation, all Petri dishes were kept in the refrigerator (4 °C) for two h and incubated after at 37 °C for 24 h for bacteria growth. Antibacterial activity was evaluated by measuring the radius of the inhibition zones to the nearest millimetre (Ozkan *et al.*, 2004).

Statistical analyses

The statistical analysis was conducted using the Statistica software program (StatSoft, Inc., Tulsa, OK, USA). All the analyses were performed in triplicate. Mean, and the standard deviation was expressed. Duncan, multiple comparison tests at 95% significance level was used to evaluate the effect of different grape varieties on a different characteristic of grape seed and grape seed oils.

3. Result and discussion

Characterisation of the grape seed samples

Table 1 shows the oil and protein content of the grape seed samples from 5 different cultivars. The oil and protein content of grape seeds significantly differed according to grape cultivars and were in the range of 6.93-8.80 and 7.44-13.66%, respectively, indicating that grape seed can be considered as a good source of oil and protein. With regarding cultivars, Pinot Noir had the highest oil and protein content (8.80 and 13.66%, respectively). Oil content of Pinot noir and Okuzgozu cultivars were reported as 17.80, and 19.60% by Göktürk Baydar and Akkurt (2001). Their results were higher than our studies. Tangolar *et al.* (2009) reported the oil content of the Okuzgozu as 11.13%. In a study (Beveridge *et al.*, 2005), the oil content of the Syrah, Merlot, Cabernet Franc, and Pinot Noir were reported as 10.8, 10.5, 13.6 and 10.7% respectively. Their results were comparable to our study. The differences between the oil content in the same varieties can be explained by different extraction techniques, solvent types and location and maturity stages.

Total bioactive compounds of the grape seeds samples are presented in Table 1. Total phenolic content and total flavonoid contents significantly differed according to grape cultivars and found to be 4,397-5,804 and 254-1,436 mg/kg respectively, indicating that the seed of these grape cultivars showed a high level of phenolic content and should be considered as a potential source of phenolics. Therefore, the grape seed has potential as a natural health beneficial source (Cotea *et al.*, 2018). High level of the phenolic content of grape seed was also be reported from various studies (Peixoto *et al.*, 2018; Zhu *et al.*, 2019).

Table 2 presents the mineral composition of the grape seed samples. According to the table, the grape cultivars significantly affected the mineral composition. Ca showed as a major mineral in grape seed samples and ranged from 5,333.40 to 7,337.10 mg/kg. Okuzgozu cultivar showed higher Ca content than that of the other cultivars.

Characterisation of cold pressed grape seed oil samples

The oil yields of cold pressing processes for Okuzgozu, Pinot Noir, Syrah, Merlot and Cabernet varieties were found to be 41.84, 43.92, 42.15, 45.50% respectively. In a study, the oil yield values of cold pressing and solvent extraction processes were reported to be 36.84 and 71.29%, respectively (Yilmaz and Guneser, 2017). In their study, oil yield of cold pressing extraction was consisted with our study, however their solvent extraction yield was higher than our results.

Fatty acid composition

Table 3 shows the fatty acid composition of cold pressed grape seed oil samples. As can be seen, the distribution of fatty acids differed significantly according to grape varieties (P<0.05). Regardless of the grape varieties, linoleic acid showed as a major fatty acid in all samples with a percentage ratio of 48.1-69.13%. Cabernet Franc showed higher linoleic acid content while Syrah exhibited the lowest linoleic acid content. Our results have shown compatibility with previously published studies (Beveridge *et al.*, 2005; Boso *et al.*, 2018; Hanganu *et al.*, 2012; Lachman *et al.*, 2015). In the literature, the linoleic acid content of the grape seed oil showed a wide range of variability (47.64-78.1%) (Akin and Citil, 2012; Lachman *et al.*, 2015).

The fatty acid composition of the Okuzgozu cultivars was also studied by Göktürk Baydar and Akkurt (2001) and Tangolar *et al.* (2009). Similarly to our studies, the linoleic acids content of their studies was reported as 66.30 and 65.81% respectively. Beveridge *et al.* (2005) reported the linoleic acids content of Pinot Noir, Syrah, Merlot and Cabernet cultivars as 70.13, 70.15, 73.23 and 70.28% respectively. Their results were higher than our studies. The differences between our results and previously published studies could be due to different extraction type, variety, cultivation procedures and location (Boso *et al.*, 2018; Wada *et al.*, 2018).

Table 1. Oil and protein, and bioactive compounds content of grape seed samples (dry matter).[1]

Sample name	Oil (%)	Protein (%)	Total phenolics (mg/kg)	Flavonoid (mg/kg)
Okuzgozu	7.00±0.50^{b}	10.32±0.41^{b}	5,535.69±6.34^{d}	1,436.67±7.09^{a}
Pinot Noir	8.80±0.60^{a}	13.66±0.23^{a}	5,804.29±4.12^{a}	346.90±4.22^{d}
Merlot	6.93±0.84^{b}	8.04±0.35^{c}	4,397.93±3.77^{e}	403.67±2.52^{c}
Syrah	7.34±0.65^{b}	7.66±0.17^{cd}	5,558.42±3.76^{c}	254.00±3.37^{e}
Cabernet Franc	7.80±0.8^{ab}	7.44±0.19^{d}	5,754.82±4.79^{b}	691.87±2.20^{b}

[1] Different lower case letter in the same column shows statistical differences (P<0.05).

Table 2. Mineral contents of grape seeds (mg/kg).[1]

Sample name	Macro elements				
	P	K	Ca	Mg	S
Okuzgozu	2,604.38±359[a]	3,408.64±707[bcd]	7,337.10±879[a]	1,902.14±227[a]	1,282.49±177[d]
Syrah	2,768.19±205[a]	2,897.09±295[d]	6,444.76±618[ab]	1,357.08±128[b]	5,184.58±288[b]
Cabernet Franc	2,616.93±438[ab]	6,310.79±657[a]	5,333.40±227[c]	1,388.22±101[b]	1,265.11±102[d]
Pinot Noir	2,580.75±117[a]	4,199.50±92[b]	5,528.37±198[c]	1,361.61±43[b]	6,089.09±97[a]
Merlot	2,128.04±93[b]	3,392.01±179[c]	5,976.06±179[b]	1,071.70±36[c]	4,032.25±20[c]
	Micro elements				
	Fe	Zn	Mn	B	Cu
Okuzgozu	35.92±8.73[c]	12.07±2.27[ab]	16.55±1.97[ab]	22.14±4.50[a]	11.43±2.27[ab]
Syrah	49.34±6.16[bc]	13.38±2.10[ab]	10.73±1.83[b]	13.60±1.06[b]	11.66±0.52[b]
Cabernet Franc	94.43±31.23[a]	15.92±2.41[a]	10.51±8.96[abc]	19.11±1.64[a]	12.26±2.30[ab]
Pinot Noir	46.79±3.50[bc]	10.59±0.72[c]	5.92±0.60[c]	19.17±2.93[a]	12.53±0.50[a]
Merlot	53.76±4.45[b]	12.03±0.54[b]	16.90±1.34[a]	13.32±0.31[b]	13.27±0.77[a]

[1] Different lower case letter in the same column shows statistical differences (*P*<0.05).

Table 3. Fatty acid composition of grape seed oils (%).[1,2]

Fatty acids	Okuzgozu	Syrah	Cabernet Franc	Pinot Noir	Merlot
Saturated fatty acid					
C8:0	Nd	Nd	Nd	Nd	Nd
C10:0	Nd	Nd	Nd	Nd	Nd
C12:0	0.05±0.02[b]	0.41±0.03[a]	0.05±0.02[b]	Nd	0.03±0.02[bc]
C14:0	0.10±0.02[b]	0.14±0.04[ab]	0.13±0.03[ab]	0.18±0.02[a]	0.15±0.02[ab]
C16:0	9.98±0.04[e]	14.82±0.04[b]	10.16±0.04[d]	10.30±0.04[c]	14.98±0.05[a]
C17:0	0.16±0.02[c]	0.22±0.02[a]	0.08±0.03[d]	0.24±0.03[a]	0.20±0.03[ab]
C18:0	4.00±0.05[c]	5.36±0.04[b]	3.61±0.04[d]	3.96±0.05[c]	6.97±0.04[a]
C20:0	0.39±0.13[c]	0.40±0.02[c]	0.60±0.03[a]	0.52±0.02[b]	0.37±0.03[d]
C22:0	0.03	Nd	Nd	Nd	Nd
∑SFA	14.69±0.06[d]	21.35±0.05[b]	14.63±0.03[d]	15.20±0.05[c]	22.70±0.04[a]
Unsaturated fatty acid					
C16:1	0.45±0.03[C]	0.80±0.03[A]	0.43±0.04[C]	0.61±0.03[B]	0.42±0.02[C]
C17:1	0.08±0.02[A]	Nd	0.04±0.01[B]	0.04±0.02[B]	0.05±0.02[AB]
C18:1 (n-9)	21.30±0.05[A]	28.94±0.04[A]	15.31±0.05[E]	19.89±0.04[D]	22.74±0.04[B]
C18:2 (n-6)	63.13±0.03[C]	48.78±0.04[E]	69.13±0.03[A]	63.80±0.05[B]	53.70±0.05[D]
C18:3 (n-3)	0.24±0.05[AB]	0.09±0.06[C]	0.30±0.03[A]	0.23±0.07[AB]	0.17±0.04[BC]
C20:1	0.07±0.03[C]	0.04±0.02[C]	0.16±0.02[B]	0.23±0.03[A]	0.22±0.02[A]
C22:1	0.04	Nd	Nd	Nd	Nd
∑MUFA	21.94±0.04[C]	29.78±0.03[A]	15.94±0.04[E]	20.77±0.04[D]	23.43±0.04[B]
∑PUFA	63.37±0.02[C]	48.87±0.03[E]	69.43±0.03[A]	64.03±0.03[B]	53.87±0.03[D]
∑UFA	85.31±0.03[A]	78.65±0.04[D]	85.37±0.03[B]	84.80±0.05[C]	77.30±0.05[E]

[1] Different letters in lowercase (a, b, c, d, and e) show the significant differences among samples' saturated fatty acids (*P*<0.05); different letters in capital (A, B, C, D, and E) show the significant differences among samples' unsaturated fatty acids (*P*<0.05).
[2] MUFA = mono unsaturated fatty acid; Nd = nonidentified; PUFA = poly unsaturated fatty acid; SFA = saturated fatty acid; UFA = unsaturated fatty acid.

Oleic acid was identified as the second major fatty acid and constituted almost the whole part of the monounsaturated fatty acid content. The oleic acid content of the samples significantly differed and ranged between 15.31 and 28.94%. Syrah and Cabernet Franch exhibited the highest and lowest percentage oleic acid level respectively. Palmitic acid was the third major fatty acid and ranged from 9.98 to 14.98%. Palmitic acid constituted an important percentage of saturated fatty acid content. Another saturated fatty acid, stearic acid, showed a ratio higher than 1%. Higher content of the C 18:0 were obtained from *Syrah* varieties. Fatty acid of the myristic, palmitoleic, and arachidic acids showed minor content in all varieties.

PUFA, MUFA, and SFA level significantly differed according to grape seed varieties ($P<0.05$). In samples, the PUFA level (48.87-69.43%) was higher than the SFA (14.63-22.70%) and MUFA (15.94-29.78%) level. Linoleic acid is the precursor of the arachidonic acid and an essential component of the cell membrane. It reduces low density lipoprotein content and, prevents diabetes and some types of cancer. Therefore the results of the fatty acid composition indicated that grape seed oil should be consumed due to higher linoleic acid and lower SFA content (Boso *et al.*, 2018).

The individual sterol composition

Table 4 presents the percentage sterol composition of the grapeseed oil samples. β-sitosterol was the major sterol (61.60-69.54%) in all varieties followed by, stigmasterol (9.21-16.71%), campesterol (8.80-12.18) and Δ5-avenasterol (2.30-2.79). The grape varieties significantly affected the percentage distribution of the sterols ($P<0.05$). Pinot Nair showed the highest β-sitosterol content while Cabernet Franc exhibited the lowest β-sitosterol level. The previously published studies (Beveridge *et al.*, 2005; Pardo *et al.*, 2009) reported similar results related to the percentage distribution of sterol of grape seed oil. The plant phytosterols have attracted considerable attention due to the reduction of arteriosclerosis. Pardo *et al.* (2009) reported that grape seed oil had higher total sterol content than some vegetable oils including sunflower oils. According to our results and these studies, the grape seed oil was a good source of β-sitosterol. β-sitosterol shows various functional and health beneficial properties such as antioxidant, immunomodulatory, antimicrobial, angiogenic, antidiabetic (Bin Sayeed *et al.*, 2016). Therefore, grapeseed oil can be considered as a good source of nutrient for lowering cardiovascular disease due to high linoleic acid and β-sitosterol level.

Physicochemical analysis

Table 5 presents the results of the physicochemical analysis of the grapeseed oils. All results of the physicochemical parameters statistically differed from sample to sample. FFA value of the samples ranged between 0,67-2.74%. Okuzgozu showed the highest FFA (2.74%) while Cabernet Franc showed the lowest value (0.67%). The FFA results of the other samples were found as 0.67-2.74%. The peroxide value of the samples ranged from 10.45 to 22.03 meqO_2/kg. According to the Codex Standard for vegetable oils (FAO/WHO, 2015), FFA and PV value must be lower than 2% and 15 meqO_2/kg respectively.

Regarding both FFA and PV value, Okuzgozu, Syrah, and Merlot samples exceeded the permitted level (FAO/WHO, 2015). Several factors such as pretreatment process, extraction type, and seed quality affect the FFA and PV values of vegetable oils. In this study, the high level of FFA can be explained by a greater lipase activity in these seeds (Costa *et al.*, 2019; Khoddami *et al.*, 2014). The antioxidant compounds levels could also affect PV value (Costa *et al.*, 2019). It was reported that some pretreatment process such as roasting increased the extraction yield of natural antioxidants and produced Maillard reaction products acting as antioxidant properties (Durmaz and Gökmen, 2010; Yilmaz *et al.*, 2015). In our studies roasting and other pretreatment process was not applied. In our study, the lower stability of the cold press grape seed oil compared to cold press oil obtained from literature could be due to some pretreatment process applied their studies (Yilmaz *et al.*, 2015).

Table 4. Percentage sterol contents of grape seed oils.[1]

Samples	Cholestrol	Brassicasterol	Campesterol	Stigmasterol	β-sitosterol	Delta-5-avenasterol	Delta-7-stigmastenol	Delta-7-avenasterol
Okuzgozu	1.21±1.02^{a}	2.12±0.01^{a}	9.37±0.14^{d}	11.99±0.06^{c}	64.97±0.08^{c}	2.73±0.00^{b}	0.75±0.01^{c}	0.67±0.03^{a}
Syrah	0.76±0.750^{b}	0.42±0.00^{c}	8.80±0.11^{e}	9.21±0.11^{e}	66.20±0.21^{b}	2.79±0.00^{a}	0.48±0.02^{d}	0.41±0.00^{c}
Cabernet Franc	0.29±0.285^{c}	0.75±0.00^{b}	10.78±0.19^{c}	16.71±0.11^{a}	61.60±0.42^{d}	2.74±0.00^{ab}	0.95±0.020^{b}	0.52±0.00^{b}
Pinot Noir	0.17±0.175^{e}	0.13±0.00^{d}	11.59±0.05^{b}	9.89±0.04^{d}	69.54±0.38^{a}	2.45±0.02^{c}	0.32±0.03^{e}	0.15±0.04^{d}
Merlot	0.24±0.230^{d}	0.10±0.00^{d}	12.18±0.02^{a}	12.52±0.10^{b}	64.80±0.56^{c}	2.30±0.02^{d}	1.21±0.04^{a}	0.47±0.01^{cb}

[1] Different lower case letter in the same column shows statistical differences ($P<0.05$).

Table 5. Physicochemical properties of grape seed oils.[1]

	Grape varieties				
	Okuzgozu	Syrah	Cabernet Franc	Pinot Noir	Merlot
Free fatty acidity (%)	2.74±0.01^{a}	2.57±0.02^{b}	0.67±0.01^{e}	1.45±0.01^{d}	2.12±0.00^{c}
Acid number (mg KOH/g oil)	5.49±0.02^{a}	5.16±0.05^{b}	1.34±0.02^{e}	2.91±0.02^{d}	4.26±0.01^{c}
Peroxide values (meqO_2/kg)	19.31±1.40^{a}	20.43±3.85^{a}	11.96±0.19^{b}	10.45±0.71^{b}	22.03±0.35^{a}
Antioxidant (µmol trolox/g oil)	0.278±0.00^{c}	0.255±0.00^{c}	0.289±0.00^{b}	0.302±0.00^{a}	0.152±0.00^{d}
Tocopherol (mg/100 g)	11.595±2.89^{a}	2.15±0.07^{d}	3.89±0.05^{b}	2.04±0.02^{d}	2.95±0,00^{c}

[1] Different lower case letter in the same column shows statistical differences (*P*<0.05).

The negative correlation (-0.74) was observed between peroxide and antioxidant capacity values, meaning that the samples with a higher antioxidant capacity showed a lower peroxide value. The antioxidant capacity value of the samples changed between 0.152-0.302 µM TE, indicating that cold press grape seed oil had a considerable antioxidant capacity. The antioxidant properties could be due to some hydrophilic and lipophilic compounds such as phenolic compounds, tocopherols, and sterols (Ben Mohamed *et al.*, 2016; Hernández-Jiménez *et al.*, 2009). Ben Mohamed *et al.* (2016) reported antioxidant capacity as 0.9-8.2 µmol Trolox/g oil, respectively. According to their results, antioxidant capacity was higher than our results. Zhao *et al.* (2017) found the DPPH for grape seed oils as 35.51-59.09 µM TE. They reported that grape seed oil had a higher antioxidant capacity than rice bran oils. Harbeoui *et al.* (2018) reported that grape seed oil showed comparable antioxidant capacity with BHT. The different findings in literature might have resulted from different extraction methods, cultivation technique and antioxidant capacity methods.

Antibacterial activity

Table 6 shows the antibacterial activity of the cold pressed grape seed oil samples on *S. aureus* (ATCC 2592), *Salmonella* Enteritidis (ATCC13076), *E. coli* (ATCC25922). Inhibition zone diameters were used to evaluate the antibacterial activity of the oil samples. According to Table 6, all samples except Pinot Noir showed the antibacterial effect on selected bacteria, and their antibacterial activity significantly differed according to varieties (*P*<0.05). Inhibition zone diameter was 6.99-8.60 mm for *S. aureus* (ATCC 2592), indicating that all samples showed the antibacterial effect on *S. aureus* (ATCC 2592). Merlot and Pinot Noir showed the highest and lowest antibacterial effect on *S. aureus* (ATCC 2592).

Regarding the *Salmonella* Enteritidis (ATCC13076), all samples except Pinot Noir showed an antibacterial effect. Inhibition zone diameters were ranged from 4.24 mm to 8.66 mm. Merlot exhibited the highest antibacterial effects. With regarding of *E. coli* (ATCC25922), inhibition zone diameters were obtained as 7.26-8.31 mm, indicating that all samples showed the antibacterial effect on selected bacteria in a different ratio (*P*<0.05).

Table 6. Antimicrobial effect of cold pressed grape seed oil against *Staphylococcus aureus* (ATCC 2592), *Salmonella enterica* subsp. *enterica* serovar Enteritidis (ATCC 13076), *Escherichia coli* (ATCC 25922).[1]

Grape varieties	Inhibition zone diameter (mm)		
	S. aureus (ATCC 2592)	*Salmonella* Enteritidis (ATCC 13076)	*E. coli* (ATCC 25922)
Okuzgozu	7.08±0.05^{c}	7.03±0.19^{c}	7.4±0.01^{b}
Syrah	7.5±0.02^{b}	7.87±0.48^{b}	7.75±0^{ab}
Cabarnet Franc	7.58±0^{b}	7.35±0.07^{bc}	7.26±0^{b}
Pinot Noir	6.99±0.05^{c}	4.24±0d	8.31±0.04^{a}
Merlot	8.6±0.19^{a}	8.66±0.03^{a}	7.48±0.45^{b}

[1] Different lower case letter in the same column shows statistical differences (*P*<0.05).

In a similar to our study, Berradre *et al.* (2016) reported that grape seed oil showed antibacterial activity in a different ratio against to *Bacillus cereus*, *S. aureus*, *Pseudomonas aeruginosa*, and *E. coli*. Xuan *et al.* (2018) reported that grape seed oil showed higher antibacterial activity on *S. aureus* and *E. coli* than some other vegetable oils. The antibacterial activity of the grape seed oil can be explained by some phenolic compounds such as resveratrol (Garavaglia *et al.*, 2016). Therefore, our study suggested that grape seed oils could be evaluated as a natural antimicrobial agent.

4. Conclusions

In this study, effect of different grape varieties on some quality parameters of grape seed and grape seed oil was investigated. This study showed that grape varieties significantly affected both grape seed and grape seed oil quality parameters. Linoleic acid was the major fatty acid in all varieties and its level changed according to grape varieties. This results suggested rape seed oil should be consumed because of the high PUFA content and selection of grape varieties for oil source was important criteria for health aspect. FFA and PV value was significantly affected from grape varieties. All cold press oil samples showed antibacterial effect. This study recommended that valorisation cold press grape seed oil in a diet could be beneficial for health due to their high PUFA and antioxidant contents.

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