

# Fatty Acid Composition, Total Phenolic Content and Antioxidant Activity of Grape Seed Oils Obtained by Cold- Pressed and Solvent Extraction

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## ABSTRACT

**Aim:** The aim of this study was to determine the influence of two different extraction methods on the fatty acid composition, total phenolic content and antioxidant activity of grape seed oils obtained from 5 different varieties. **Materials and Methods:** The seeds of the Syrah, Merlot, Sangiovese, Cabernet Sauvignon, Sauvignon Blanc grape varieties were used in this study. Oil extraction was carried out by Soxhlet extraction and cold-pressed extraction methods. **Results:** The most abundant fatty acids in the grape seed oils that were obtained were linoleic acid, oleic acid and palmitic acid ranging from 64.80 to 68.64%, 18.41 to 21.58% and 7.08 to 8.23%, respectively. The linoleic and palmitic acid values that were determined in the grape seed oils obtained by solvent extraction were higher than those for the grape seed oils obtained from the cold-pressed samples. The total phenolic content and antioxidant activity of the grape seed oils varied between 115.68 and 317.71 mg GAE/kg, 7.14 and 34.46% as DPPH radical scavenging activities, respectively. **Conclusion:** The total phenolic content and antioxidant activity values for the grape seed oils obtained by solvent extraction were higher than those determined in the grape seed oils obtained by cold-pressing.

**Key words:** Grape seed oil, Fatty acid composition, Phenolic content, Antioxidant activity, Extraction method.

## INTRODUCTION

Grapes, the berries of *Vitis vinifera* L. ssp *sativa*, are one of the major fruit crops cultivated in many areas of the World.<sup>1,2</sup> Turkey has production of 4 million tons of grapes, as the world's sixth-largest grape-producing country according to the data in 2017.<sup>3</sup> During the production of some products such as wine, vinegar, grape juice from grapes are obtained large quantity pomace.<sup>4,5</sup> Grape pomace accounts for 20% of grape and it is composed of seeds, skin and stems.<sup>6</sup> Grape seeds, corresponding to 38-52% on a dry matter basis, are significant agricultural and industrial wastes.<sup>7</sup> Grape seeds contain from 8 to 20% of oil depending on the extraction methods and variety.<sup>8</sup> Grape seed oils include about poly- and monounsaturated fatty acids, as in the amount of 90%, par-

ticularly of linoleic acid (58-78%, C18:2) followed by oleic acid (3-15%, C18:1) and minor amounts of saturated fatty acids (10%).<sup>6,8</sup> Polyunsaturated fatty acids such as linoleic acid are referred to in the literature as desirable compounds in the human diet because of their effect in reducing the risk of coronary heart diseases and cancer.<sup>2</sup> On the other hand, oleic acid is considered to reduce the levels of low density lipoproteins and prevent arteriosclerosis.<sup>1,2</sup> Furthermore, grape seed oil contains minor components such as phenolic compounds.<sup>6,9</sup> The benefits of phenolic compounds on human health are correlated with their antioxidant activity.<sup>10</sup> Phenolic compounds are reported to be beneficial for oil conservation due to their increasing oil oxida-

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tive stability.<sup>1,6</sup> Grape seed oil has been recognized as a high-quality oil for infants and elderly people.<sup>5,11</sup> Soft fruity taste, high smoking point (210-230°C), with not a significant increase in its viscosity when it is used as a frying oil, being digestible to great extent are the main properties that describe the good qualities of grape seed oil.<sup>12,13</sup> Grape seed oil is obtained from cold-pressing, solvent extraction (Soxhlet), instrumental methods (microwave, ultrasound, supercritical fluid extraction), enzyme assisted aqueous extraction, infrared, X ray absorption.<sup>13-17</sup> The cold-pressing method is a method of oil extraction without heat and chemical treatment and therefore, it may keep health beneficial components such as antioxidative phenolic compounds.<sup>6,18</sup> Even though the yield is lower when it is compared to conventional solvent extraction, it is not harmful for the health of the product and it is a safer method due to the fact that there is no solvent residues in the oil.<sup>1</sup>

To our knowledge, there is no study about the effects of different extraction methods on the fatty acid composition, total phenolic content and antioxidant activity of grape seed oil. Therefore, the aim of this study was to determine the influence of two different extraction methods on the fatty acid composition, total phenolic content and antioxidant activity of grape seed oils obtained from 5 different varieties.

## MATERIALS AND METHODS

The seeds of the Syrah, Merlot, Sangiovese, Cabernet Sauvignon, Sauvignon Blanc grape varieties were used in this study. The grapes were collected at harvesting time (13-26 July) from the commercial wine grapes production vineyard of the province of Hatay in the Eastern Mediterranean Region in Turkey in 2016. The grapevine establishment is located at 36° 25.5' K, 36° 15.7' D and its altitude from the sea is 114 m. The cultivars were grafted on 41B rootstock and embedded in 2X1 m spaces in 2008 and trained in a single guyot system.

### Chemicals

All the chemicals that were used in the experiments were of analytical grade. Gallic acid, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's reagent, sodium carbonate, methanol, n-hexane, diethyl ether, were obtained from Merck (Germany) and Sigma-Aldrich (Germany). The fatty acid methyl ester (FAME) mixes were obtained from Supelco (Bellefonte, USA).

### Water and Oil Content

The water content of the grape seeds were calculated based on the method by Rombaut *et al.*<sup>6</sup> The oil content of the grape seeds was determined based on the

method described by the American Oil Chemists' Society (AOCS) Official Methods Am 2-93<sup>19</sup> by the Soxhlet extraction method using n-hexane at 80°C for 6 h.

### Oil Extraction

Oil extraction was carried out by Soxhlet extraction and cold-pressed extraction methods. Oil extraction by the Soxhlet method was as mentioned in oil content.

### Cold-pressed Extraction

Oil extraction by the cold-pressed method was performed on a screw press (Kocmaksan, Turkey). The pressing was conducted without external heating. The oily phase was centrifuged at 5 min, 3000 rpm and room temperature to separate the oil from the sediment.

Both the Soxhlet oil and the cold-pressed oil were stored at +4°C until analyses which were duplicated.

### Fatty Acid Composition

The fatty acid methyl esters (FAMES) were prepared from the grape seed oils using a cold transmethylation by shaking a solution of 0.2 g oil and 3 ml of hexane with 0.4 ml of 2 mol/L methanolic potassium hydroxide.<sup>20</sup>

The analysis of the FAME was performed by an Agilent gas chromatography system (Agilent 6850, USA) using a hydrogen flame ionization detector (FID) and a capillary column DB23 (60 m length 9 0.25 mm i.d. and 0.25 µm film thickness). The temperatures of the detector and the injector were set at 230 and 280°C, respectively. Helium was employed as carrier gas with a flow rate of 1 ml/min and the split ratio was 1:50. The injection volume was 1 µl. The results were expressed as a relative area percentage of total fatty acid methyl esters. The fatty acids were determined by comparing their retention times to those of the reference compounds.

### Total Phenolic Contents

The total phenolic contents (TPC) of the grape seed oils were calculated by using the Folin ciocalteu colorimetric method according to the method described by Bail *et al.*<sup>8</sup> with some modifications. For the extraction of the phenols from oil 80:20, methanol: water (3ml) was added to 1 g of each oil followed by vortex for two min and centrifuged for 5 min at 3000 rpm. The extraction was carried out three times. All the methanolic extracts were combined and concentrated until dryness. After the extraction process, 0.2 ml of the phenolic extract was mixed with 1a ml of the Folin-Ciocalteu reagent. 3 ml of the saturated sodium carbonate solution (10%) was added to this mixture. After vigorous shaking, the volume of the solution was increased to 10 ml with distilled water. The mixture was left to stand for 30 min

in the dark at room temperature. The absorbance was recorded at 765 nm in a UV-Vis spectrophotometer (Hitachi U-1900, Japan). Quantification was carried out using a standard curve built with 25-50-100-200 mg/l prepared in an aqueous solution of methanol (70%). The results were expressed as milligrams of gallic acid equivalents per kilogram of oil (mg GAE/kg).

### Antioxidant Activity

The antioxidant activity (free radical scavenging capacity) of the grape seed oil phenolic extracts was determined by using the method of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical according to the procedure of Brand Williams *et al.*<sup>21</sup> with some modifications. Briefly, 1 ml of extracts was diluted with 1.9 ml DPPH methanolic solutions. After 60 min of incubation in the dark at room temperature, the absorbance was measured at 515 nm against a blank (MeOH).

The percentage of inhibition was calculated from the following equation:

$$\% \text{Inhibition (DPPH)} = \left[ \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}} \right] \times 100$$

### Statistical analysis

The experiment was set up as a two-factor completely randomized design. Each treatment included 3 replicates. The variance analyses of the obtained data were made by using the Mstat-C computer software. The differences between the averages were evaluated by a Tukey test at 0.05.<sup>22</sup>

## RESULTS AND DISCUSSION

### Water and Oil Content

The water and oil contents of the grape seeds with different varieties are given in Table 1. As shown in Table 1, the water content of the grape seeds ranged from 7.94 (Sauvignon Blanc) to 9.94% (Sirah). The results indicated that the water content of the grape seeds showed significant differences depending on the variety ( $P < 0.05$ ). The oil content of the grape seed was around 7 to 20%.<sup>23</sup> The oil contents of the grape seeds were between 4.95% (Sangiovese) and 7.77% (Sirah). Our results were lower than those obtained by Gökürk Baydar and Akkurt,<sup>4</sup> Lachman *et al.*<sup>2</sup> and Apaydın *et al.*<sup>5</sup> In another study on Turkish grape seeds, the results were higher, varying between 12.35 in Kalecik Karası and 16.00% in Hasandede.<sup>24</sup> The differences between the values of grape seed oil content depended on the variety and the cultivation conditions<sup>20</sup> and extraction methods.<sup>8</sup> Lutterodt *et al.*<sup>1</sup>, stated that oil yield with the

cold-pressed method was lower than that with solvent extraction.

### Fatty acid composition

The fatty acid composition of the grape seed oils obtained by the cold-pressed and solvent extraction methods were shown in Table 2. Linoleic (C18:2), oleic (C18:1), palmitic (C16:0) and stearic (C18:0) were the major fatty acids in the grape seed oils. The minor fatty acids were palmitoleic (C16:1), linolenic (C18:3), arachidic (C20:0) and gadoleic (C20:1) acid. Linoleic acid was the most abundant fatty acid in all the grape seed oils, ranging from 64.80% in Sirah to 68.64% in Merlot. The linoleic acid content of the grape seed oils showed significant ( $P < 0.05$ ) variations across the varieties (except for the Sauvignon Blanc and Sangiovese variations). Furthermore, the linoleic acid content of all the grape seed oils was significantly ( $P < 0.05$ ) influenced by the extraction methods. The results indicated that the linoleic acid content of the grape seed oils obtained by the solvent extraction (67.54%) method was higher than that of the grape seed oils obtained by the cold-pressed method (66.16%). The interaction between the variety and the extraction method was found to be significant among all the fatty acids of the studied grape seed oils.

A-D express significant statistical differences between cultivars. X-Y letters express significant statistical differences between extraction methods. Different small letters express significant statistical differences in the interaction of cultivars by the extraction methods of X (Tukey test  $P < 0.05$ ), N.S: Not significant.

Tangolar *et al.*<sup>25</sup> stated that the ranges of fatty acids in the grape seeds were 62.5 in Alicante Bouschet to 69.24% in Muscat of Hamburg varieties for linoleic acid. Additionally, Fernandes *et al.*<sup>7</sup> reported that the linoleic acid content of Portuguese grape seed oils was in the range of 63.0 - 73.1%. Our results were in good agreement with those obtained by Tangolar *et al.*<sup>25</sup> and Fernandes *et al.*<sup>7</sup> The oleic acid content in our study varied between 18.41% in Sauvignon Blanc and 21.58% in Sirah.

**Table 1: Water and oil contents of grape seeds.**

Cultivar	Oil Content (%)	Water content (%)
Sirah	7.77 a*	9.94 c
Merlot	5.65 bc	9.10 bc
Sangiovese	4.95 c	8.77 ab
Cabernet Sauvignon	5.65 bc	8.08 a
Sauvignon Blanc	7.10 ab	7.94 a
D%5	2.11	0.85

\*: The differences between the means marked with different letters ( $p < 0.05$ )

**Table 2: Fatty acid compositions of grape seed oils.**

Cultivar	Extraction method	Palmitic (C16:0)	Palmitoleic (C16:1)	Stearic (C18:0)	Oleic (C18:2)	Linoleic (C18:2)	Linolenic (C18:3)	Arachidic (C20:0)	Gadoleic (C20:1)
Sirah	Solvent	8.270 b	0.247 c	4.110 d	21.567 a	64.977 f	0.327 bc	0.137 f	0.170 a
	Cold-pressed	8.190 b	0.223 d	4.103 d	21.593 a	64.613 f	0.300 d	0.127 f	0.160 ab
<b>Mean</b>		<b>8.230 A</b>	<b>0.235 C</b>	<b>4.107 C</b>	<b>21.580 A</b>	<b>64.795 D</b>	<b>0.313 CD</b>	<b>0.132 C</b>	<b>0.165 A</b>
Merlot	Solvent	7.513 e	0.210 d	3.660 c	17.350 f	70.500 a	0.347 b	0.207 b	0.167 a
	Cold-pressed	7.247 f	0.313 b	3.843 ef	20.937 b	66.770 d	0.317 cd	0.203 b	0.160 ab
<b>Mean</b>		<b>7.380 C</b>	<b>0.262 B</b>	<b>3.752 E</b>	<b>19.143 C</b>	<b>68.635 A</b>	<b>0.332 B</b>	<b>0.205 A</b>	<b>0.163 A</b>
Sangiovese	Solvent	7.217 f	0.337 a	3.733 fg	20.493 c	67.387 c	0.380 a	0.173 cd	0.163 ab
	Cold-pressed	6.947 g	0.263 c	3.993 de	21.253 ab	66.783 d	0.313 cd	0.143 ef	0.170 a
<b>Mean</b>		<b>7.082 D</b>	<b>0.300 A</b>	<b>3.863 D</b>	<b>20.873 B</b>	<b>67.085 B</b>	<b>0.347 A</b>	<b>0.158 B</b>	<b>0.167 A</b>
Cabernet Sauvignon	Solvent	8.470 a	0.123 e	5.127 b	17.123 f	67.800 b	0.300 d	0.253 a	0.120 e
	Cold-pressed	7.960 c	0.213 b	4.500 c	20.100 d	65.550 e	0.313 cd	0.157 de	0.150 bc
<b>Mean</b>		<b>8.215 A</b>	<b>0.168 D</b>	<b>4.813 B</b>	<b>18.612 D</b>	<b>66.675 C</b>	<b>0.307 D</b>	<b>0.205 A</b>	<b>0.135 B</b>
Sauvignon Blanc	Solvent	7.623 de	0.080 f	5.857 a	18.537 e	67.030 cd	0.317 cd	0.240 a	0.127 de
	Cold-pressed	7.730 d	0.117 e	5.983 a	18.280 e	67.060 cd	0.330 bc	0.177 c	0.137 cd
<b>Mean</b>		<b>7.677 B</b>	<b>0.098 E</b>	<b>5.920 A</b>	<b>18.408 D</b>	<b>67.045 B</b>	<b>0.323 BC</b>	<b>0.208 A</b>	<b>0.132 B</b>
<b>Extraction method</b>	Solvent	<b>7.819 X</b>	<b>0.199 Y</b>	<b>4.497</b>	<b>19.014 Y</b>	<b>67.539 X</b>	<b>0.334 X</b>	<b>0.202 X</b>	<b>0.149 Y</b>
	Cold-pressed	<b>7.615 Y</b>	<b>0.226 X</b>	<b>4.485</b>	<b>20.433 X</b>	<b>66.155 Y</b>	<b>0.315 Y</b>	<b>0.161 Y</b>	<b>0.155 X</b>
D%5 cultivar		0.109	0.011	0.095	0.225	0.225	0.012	0.012	0.008
D%5 Extraction method		0.048	0.005	N.S.	0.099	0.099	0.005	0.005	0.004
D%5 Cultivar X extraction method		0.174	0.017	0.151	0.359	0.359	0.019	0.019	0.013

There were significant differences among the varieties in terms of the oleic acid content of grape seed oils (except for Sauvignon Blanc and Cabernet Sauvignon). The oleic acid content of all grape seed oils was significantly ( $P<0.05$ ) influenced by the extraction methods. The oleic acid content of the grape seed oils that were obtained by the cold-pressed method (20.43%) was higher than that of the grape seed oils obtained by the solvent extraction method (19.01%). Lutterodt *et al.*<sup>1</sup> reported values for oleic acid between 13.19% (Concord) and 21.9% (Ruby Red), Sabir *et al.*<sup>20</sup> between 18.31 (Red Globe) and 31.2% (Hönüsü), Göktürk Baydar and Akkurt,<sup>4</sup> between 17.8 (Emir) and 26.5 (Cavus).

The palmitic acid content ranged from 7.08% in Sangiovese to 8.23% in Sirah. Significant differences among the cultivars were determined in terms of the palmitic acid contents of the grape seed oils (except for the Sirah and Cabernet Sauvignon varieties). On the other hand, the variations between the palmitic acid contents of all the grape seed oils were found to be significant ( $P<0.05$ ) between the extraction methods. The palmitic acid contents of the grape seed oils that were obtained by solvent extraction (7.82%) was higher when it was compared to the values of the grape seed oils that were obtained by the cold-pressed method (7.62%). Göktürk Baydar and

Akkurt<sup>4</sup> reported palmitic acid values to vary between 6.5 and 9.7% in the Muscat of Hamburg and Rasacu varieties, respectively. Fernandes *et al.*<sup>7</sup> obtained values for palmitic acid ranging from 6.17% in the Periquita to 8.50% in Tinta Barroca varieties. Our results were in good accordance with these results. Additionally, the stearic and linolenic acid contents of the grape seed oils were in the range of 3.75 in Merlot – 5.92% in Sauvignon Blanc, 0.31 in Cabernet Sauvignon- 0.35% in Sangiovese. There were significant ( $P<0.05$ ) differences among the varieties in terms of their stearic acid contents, whereas no significant differences across the the extraction methods were found.

### **Total Phenolic Contents (TPC) and Antioxidant Activity**

As presented in Table 3, the TPC were in the range of 115.68 for Sauvignon Blanc - 317.71 mg GAE/kg for Cabernet Sauvignon. There were significant differences across the varieties in terms of the total phenolic values of all the grape seed oils. Moreover, the TPC of all the grape seed oils was significantly ( $P<0.05$ ) effected by the extraction methods. The TPC values for the grape seed oils that were obtained by solvent extraction (253.04 mg/kg) were higher than those determined in

**Table 3: Total phenolic content (TPC) and antioxidant activity of grape seed oils.**

Cultivar	Extraction method	TPC (mg/kg GAE)	DPPH radical scavenging effect (%)
Sirah	Solvent	182.590 c*	26.333 b
	Cold-pressed	148.210 de	13.280 e
<b>Mean</b>		<b>165.400 C</b>	<b>19.807 B</b>
Merlot	Solvent	148.527 de	17.517 c
	Cold-pressed	151.510 d	17.937 c
<b>Mean</b>		<b>150.018 D</b>	<b>17.727 C</b>
Sangiovese	Solvent	352.293 b	27.290 b
	Cold-pressed	177.307 c	10.410 f
<b>Mean</b>		<b>264.800 B</b>	<b>18.850 BC</b>
Cabernet Sauvignon	Solvent	452.997 a	53.593 a
	Cold-pressed	182.413 c	15.317 d
<b>Mean</b>		<b>317.705 A</b>	<b>34.455 A</b>
Sauvignon Blanc	Solvent	128.813 e	7.240 g
	Cold-pressed	102.550 f	7.037 g
<b>Mean</b>		<b>115.682 E</b>	<b>7.138 D</b>
<b>Extraction method mean</b>	Solvent	<b>253.044 X</b>	<b>26.395 X</b>
	Cold-pressed	<b>152.398 Y</b>	<b>12.796 Y</b>
D%5 Cultivar		12.191	1.155
D%5 Extraction method		5.377	0.509
D%5 Cultivar X extraction method		19.442	1.841

the grape seed oils obtained by the cold-pressed method (152.40 mg/kg). To obtain the antioxidant potential of the seed oils from different grape varieties, DPPH radical scavenging activities were measured. As shown in Table 3, the DPPH radical scavenging activities of the grape seed oils ranged from 7.14 for Sauvignon Blanc to 34.46% for Cabernet Sauvignon. Significant variations among the varieties were found in terms of the DPPH radical scavenging activities of grape seed oils.

A-D express significant statistical differences between cultivars. X-Y letters express significant statistical differences between extraction methods. Different small letters express significant statistical differences the interaction of cultivars X extraction methods (Tukey test  $P < 0.05$ ).

Furthermore, the DPPH radical scavenging activities of the grape seed oils showed significant ( $P < 0.05$ ) variations between the extraction methods. The lowest DPPH radical scavenging activities were determined in the grape seed oils obtained by the cold-pressed method (12.80%), while the highest values for the grape seed oils was obtained by solvent extraction (26.40%). Apaydin *et al.*<sup>5</sup> stated the total phenolic content values of grape seed oils to be varying between 1725 for Alicante Bouschet and 3330 mg GAE/kg for Cabernet and the antioxidant activity was found in the range between 0.106

in Shiraz and 0.19  $\mu\text{mol}$  troloks /g in Cinsault for grape seed oils. Fernandes *et al.*<sup>7</sup> reported values for DPPH radical scavenging activities to be ranging from 38.68 for Trincadeira Preta to 69.89% for Cornifesto. As previously reported by Lutterodt *et al.*<sup>1</sup> the total phenolic content and the antioxidant activity of the grape seed oils that were obtained by the cold-pressed method varied between 160 for Ruby red and 800 mg GAE/kg for Concord, 0.07 in Chardonnay and 2.22 mmol troloks /g in Concord, respectively.

## CONCLUSION

The results of this study provide experimental data for the grape seed oils obtained by the solvent and cold-pressed acquisition methods in terms of their fatty acid profiles, total phenolic content and antioxidant activity. Linoleic acid, which was the most abundant fatty acid, total phenolic content and antioxidant activity were found at the highest levels in the grape seed oils obtained by solvent extraction. On the other hand, the oleic acid values of the oils that were obtained with the cold-pressed methods was higher than those of the oils that were obtained by the solvent extraction method.

## Research Highlights

There is no study about the effects of different extraction methods on fatty acid composition, total phenolic

content and antioxidant activity of grape seed oil. The results of this study provides experimental data for the grape seed oils obtained by solvent and cold pressed in terms of fatty acid profile, total phenolic content and antioxidant activity.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**FAMES:** Fatty acid methyl esters; **TPC:** Total phenolic contents; **DPPH:** 2,2-diphenyl-1-picrylhydrazil; **GAE:** Gallic acid equivalents.

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## Summary

- Grape seed oils were extracted with cold-pressed and solvent extraction methods
- The quality characteristics such as fatty acid composition, the total phenolic content, antioxidant capacities were compared in the oil samples extracted by different methods.
- The total phenolic content and antioxidant activity values for the grape seed oils obtained by solvent extraction were higher than those determined in the grape seed oils obtained by cold-pressing.

### PICTORIAL ABSTRACT



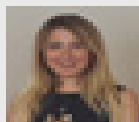
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