

Bioactive Properties, Fatty Acid Composition and Mineral Contents of Grape Seed and Oils

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Submitted for publication: October 2016

Accepted for publication: November 2016

Key words: Grape seed, varieties, oil, fatty acid, phenol, flavonoid, minerals

The oil content of grape seed samples changed between 4.53% (Adakarasi) and 11.13% (Sauvignon blanc). While the linoleic acid contents of grape seed oils ranged from 47.34% (Sangiovese) to 72.91% (Cinsaut), the oleic acid contents varied between 13.35% (Cabernet Sauvignon) and 26.30% (Sangiovese) ($p < 0.05$). Also, the palmitic acid and stearic acid contents of grape seed oils ranged from 7.15% (Cinsaut) to 16.06% (Sangiovese) and from 2.43% (Narince) to 6.55% (Sangiovese) respectively ($p < 0.05$). The flavonoid contents of the seeds changed between 263.53 (mg CE/g) and 1 706.00 (mg CE/g) (Cabernet Sauvignon). Total phenols were found to be between 6 711.14 (mg GA/g extract) (Sangiovese) and 8 818.69 (mg GA/g extract) (Narince). The linoleic contents of oils changed from between 47.34% (Sangiovese) and 72.91% (Cinsaut). The K contents of the grape seed samples changed between 4 347.80 mg/kg (Cabernet Sauvignon) and 9 492.60 mg/kg (Gamay) ($p < 0.05$). The Fe contents of seeds were found to be between 29.96 mg/kg (Narince) and 73.82 mg/kg (Sangiovese). As a result, the current study shows that grape seeds are useful for human nutrition due to their components.

INTRODUCTION

Grapevines (*Vitis vinifera* L.) are grown in the temperate and tropical regions of the world for fresh fruit, raisins, juice and wine (Winkler *et al.*, 1974; Riaz *et al.*, 2004). People in Italy, France, Spain, Turkey and Greece have benefited from the by-products of wine, vinegar, grape juice and “pekmez” (boiled grape juice). Most of the seeds (about 20% to 26% of the pomace) are produced in large quantities by wineries (Kamel *et al.*, 1985; Göktürk Baydar & Akkurt, 2001). Grape species have economic importance in both the fresh and processed fruit markets. Generally, a large amount of grape seeds are obtained from the waste of processed fruit products, like juice, jam or marmalade and boiled juice. The wastes of this industry, such as peels, seeds and pulps, represent about 50% to 60% of the raw processed fruit. Besides being a potentially valuable waste resource, they also aggravate already serious disposal problems. In recent years, grape seed has attracted attention due to the further use of the grape seeds as a potential source of other nutrients. Obviously, such utilisation would improve the utilisation of the available resources and result in the production of various products for food. On the other hand, grape seeds are

used as a source of oil, as they have nutritional and bioactive constituents (Kamel *et al.*, 1985; Göktürk Baydar & Akkurt, 2001; Hassanein & Abedel-Razek, 2009). Also, grape seeds have a high protein content (Hassanein & Abedel-Razek, 2009). They also contain 10% to 20% oil (Schester *et al.*, 1992; Laufenberg *et al.*, 2003; Akin & Altındışlı, 2011) with a high vitamin E content, which is important for human health (Barron *et al.*, 1988). Ashraf and Taylor (2004) researched the polyphenol and procyanidin compounds from grape seeds. Nakamura *et al.* (2003) studied the procyanidins of grape seed extracts, and health foods containing grape seed extract and oils. It was established that grape seed oil is rich in unsaturated fatty acids, such as linoleic acid (72% to 76%) (Citol *et al.*, 2010; Akin & Altındışlı, 2011). Generally, grape seed oil is used for salad dressings, marinades, deep frying, flavoured oils, baking, massage oil, sunburn repair lotion, hair products and hand creams (Akin & Altındışlı, 2011). Due to the importance of grape seeds as sources of oil, minerals and proteins, the aim of this study was to determine some chemical properties, fatty acids and minerals of grape seeds in eleven grape cultivars.

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Acknowledgements: The authors extend their appreciation to the International Scientific Partnership Programme (ISPP) at King Saud University for funding this research work through ISPP# 0015

MATERIALS AND METHODS

Materials

The grape fruits were obtained from the Tekirdağ Vineyard Research Centre in Turkey (Table 1). The grapes were crushed in the winery to remove the stems. The seeds were separated from the crushed grapes and washed with distilled water. The seeds were dried for in an oven for 24 h at 70°C.

Oil extraction

Oil was extracted from ground seeds with petroleum ether in a Soxhlet extractor for 6 h. The solvent (petroleum ether) was then evaporated. The obtained oil was kept in coloured bottles at -18°C before analysing.

Fatty acid composition

The oil samples (50 mg to 100 mg) were converted to their fatty acid methyl esters (FAME). The fatty acid methyl esters were identified by comparing the retention time of the samples with the appropriate fatty acid methyl ester standards (Hişil, 1998). The fatty acid methyl ester was injected into a Varian 5890 gas chromatograph with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 µm).

Determination of mineral and protein contents

Grape seed samples were dried in a drying cabinet at 70°C. About 0.5 g of sample was digested in a closed microwave system by using 5 ml of 65% HNO₃ and 2 ml of 35% H₂O₂. Then 20 ml of ultra-deionised water was added to the digested sample, and the sample was examined by ICP AES (Varian-Vista, Australia) for minerals (Skujins, 1998). The protein determination was done in a Leco combustion analyser and 6.25 was used as the conversion factor. The crude protein content of the samples was determined according to the Association of Official Analytical Chemists (AACC International, 1999).

Determination of anthocyanins and total phenolic and flavonoid contents

The anthocyanin contents of the plant samples were analysed according to the method of Ticconi *et al.* (2001). After 0.5 g fresh weight (FW) was homogenised in a solution containing propanol, hydrochloric acid and water (18 : 1 : 81), the

homogenate was boiled in a water bath for 3 min and then left in darkness for 24 h at room temperature. A total of 3 mL of the supernatant was centrifuged at 6 500 rpm for 40 min. Finally, the absorbencies of the samples were measured at 535 and 650 nm.

The phenols of the plants were determined according to Madaan *et al.* (2011). Absorbances were measured at 765 nm using a UV/VIS spectrophotometer (Schimadzu, Japan) against a blank.

The total flavonoid contents (mg CE/g) of the seeds were estimated according to Dewanto *et al.* (2002). Methanol extracts were properly diluted with distilled water. Then 5% NaNO₂ solution was added to each test tube, and it was allowed to stand for five minutes. Then percent AlCl₃ solution then 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.

Statistical analyses

Analysis of variance (ANOVA) was calculated by using JMP version 9.0 (SAS Inst. Inc., Cary, N.C., U.S.A.) and the mean ± standard deviation (MSTAT C) of the results was calculated according to Püskülcü and İkiz (1989).

RESULTS AND DISCUSSION

The oil, protein, flavonoid, total phenol and total anthocyanin contents of the grape seeds are given in Table 2. The oil contents of the seeds ranged from 4.53% (Ada Karası) to 11.13% (Sauvignon blanc) ($p < 0.05$). In a previous study, the oil contents of some Turkish grape seeds were found to be between 8.09% (Dökülgün) and 13.24% (Hesap Ali) (Akın & Altındışlı, 2011). Hassanein and Abadel-Razek (2009) determined 12.0% oil in grape seed. In another study, the oil contents of grape seeds ranged between 11.6% and 19.6% (Göktürk Baydar & Akkurt, 2001; Göktürk Baydar *et al.*, 2007). The protein contents of the grape seed samples varied between 7.51% (Chardonnay) and 13.28% (Sauvignon blanc). Fazlo *et al.* (1982) reported that grape seed contained 13.64% crude protein. In addition, Kamel *et al.* (1985) determined 8.4% protein in grape seed. While

TABLE 1
Grape seeds used in experiment.

Grape varieties	Parts used	Seed amount (g)	Origin
Alphonse Lavallé	Seed	250	France
Ada Karası	Seed	250	Turkey
Sauvignon blanc	Seed	250	France
Sangiovese	Seed	250	Italy
Papaz Karası	Seed	250	Turkey
Narince	Seed	250	Turkey
Gamay	Seed	250	France
Semillon	Seed	250	France
Cinsaut	Seed	250	France
Chardonnay	Seed	250	France
Cabernet Sauvignon	Seed	250	France

TABLE 2
Chemical properties of grape seeds (dw).

Grape varieties	Crude oil (%)	Crude protein (%)	Flavonoid (mg CE/g)	Total phenolics (mg GA/g extract)	Anthocyanins (μmol/g dw)
Alfons Lavallé	8.60 ± 1.08*b	11.54 ± 0.25b	519.50 ± 3.05c	8 718.47 ± 3.41a	0.80 ± 0.04b
Ada Karası	4.53 ± 0.98d**	12.89 ± 0.53a	448.57 ± 4.97c	8 644.28 ± 3.93a	0.55 ± 0.03c
Sauvignon blanc	11.13 ± 1.27a	13.28 ± 0.12a	263.53 ± 3.61d	6 791.43 ± 4.21c	0.47 ± 0.02c
Sangiovese	10.66 ± 1.17a	11.89 ± 0.24b	967.90 ± 3.25b	6 711.14 ± 5.72c	0.31 ± 0.03c
Papaz Karası	6.26 ± 0.78c	7.85 ± 0.18c	348.30 ± 1.55d	7 556.38 ± 4.33b	0.66 ± 0.03b
Narince	9.40 ± 0.83a	7.70 ± 0.10c	647.00 ± 5.57c	8 818.69 ± 2.56a	0.86 ± 0.06b
Gamay	7.26 ± 0.91b	7.83 ± 0.13c	426.73 ± 170.39c	8 793.72 ± 2.85a	0.56 ± 0.05c
Semillon	7.20 ± 0.89b	7.99 ± 0.40c	436.47 ± 3.70c	8 346.81 ± 3.75a	0.34 ± 0.04c
Cinsaut	5.93 ± 0.67	7.74 ± 0.03	378.10 ± 3.16d	8 593.11 ± 3.41a	2.55 ± 0.06a
Chardonnay	9.26 ± 0.09a	7.51 ± 0.07c	533.50 ± 3.25c	8 765.50 ± 3.85a	0.49 ± 0.02c
Cabernet Sauvignon	10.06 ± 0.87a	7.79 ± 0.07c	1 706.00 ± 5.00a	8 229.01 ± 3.18a	0.76 ± 0.04b

* mean ± standard deviation; ** means in the same row with the same letters are not significantly different (p < 0.05)

the flavonoid contents of grape seeds varied between 263.53 (Sauvignon blanc) and 1 706.00 mg CE/g (Cabernet Sauvignon), the total phenol contents of grape seeds ranged from 6 711.14 (Sangiovese) to 8 818.69 mg GA/g extract (Narince). Also, the anthocyanin contents of seeds varied between 0.31 (Sangiovese) and 2.55 μmol/g dry weight (dw) (Cinsaut). The crude oil and protein contents of grape seeds were found to differ somewhat from the results in the literature. These differences can be due to grape cultivars, agronomic conditions and the ripening period of the grapes.

The fatty acid composition of grape seed oils is shown in Table 3. The results reveal that grape seed oils are rich in linoleic oleic and palmitic and stearic acids. While the linoleic acid contents of grape seed oils range from 47.34% (Sangiovese) to 72.91% (Cinsaut), the oleic acid contents of seed oils vary between 13.35% (Cabernet Sauvignon) and 26.30% (Sangiovese) (p < 0.05). Also, the palmitic acid and stearic acid contents of grape seed oils range from 7.15% (Cinsaut) to 16.06% (Sangiovese), and from 2.43% (Narince) to 6.55% (Sangiovese) respectively (p < 0.05). The linoleic acid was the key fatty acid in grape seed oil, followed by palmitic and oleic acids. Other fatty acids were found in small quantities. Five grape cultivar seed oils contained 6.7% to 8.9% palmitic acid, 1.1% to 5.3% stearic acid, 9.7% to 17.5% oleic acid, 69.2% to 80.5% linoleic acid, and < 0.1% palmitoleic and linolenic acids (Ohnishi *et al.*, 1990). In other study, grape seed oil was found to contain 6.7% to 9.1% palmitic acid, 13.4% to 20.7% oleic acid, and 68.1% to 78.1% linoleic acid (Yoo *et al.*, 1984). In the seed oils of red and white Italian grapes, Riccardo and Muratore (1993) determined 65.9% and 62.2% linoleic, 18.6% and 16.9% oleic, 11.6% and 10.7% palmitic, 3.8% and 3.4% stearic, and 3.5% and 2.8% myristic acid respectively. Won Young *et al.* (2000) reported that linoleic, oleic, palmitic and stearic acids were the main fatty acids of grape seed oil. The oils of grape seed cultivars contained 8.40% to 6.51% palmitic acid, 16.1% to 11.62% oleic acid, 77.59% to

72.50% linoleic acid and 3.86% to 3.07% stearic acid (Uslu & Dardeniz, 2009). Grape seed oil contained 4.1% palmitic, 10.4% stearic, 16.4% oleic and 69.3% linoleic acid (Özcan *et al.*, 2010). The results as they related to the grape seed oils were very similar to the findings in the literature. As a result, it can be stated that there is a slight variation among cultivars in fatty acid composition. It therefore is concluded that the seeds as a by-product of the grape-processing industries could be benefited for mainly edible oil and other functional components.

Table 4 shows the mineral contents of 11 grape seeds. The P contents of the seed samples varied between 2 277.65 (Papaz Karası) and 3 232.42 mg/kg (Chardonnay). In addition, while the K contents of grape seeds varied from 4 347.80 (Cabernet Sauvignon) to 9 492.60 mg/kg (Gamay), the Ca contents of seeds ranged from 5 115.58 (Chardonnay) to 8 036.76 mg/kg (Ada Karası) (p < 0.05). Also, the Mg contents of the samples were found to be between 1 249.18 (Ada Karası) and 2 073.90 mg/kg (Alfons Lavallé). In addition, the Fe contents of the grape seeds ranged from 29.96 (Narince) to 73.82 mg/kg (Sangiovese). While the Zn contents of the seeds varied from 8.27 (Narince) to 15.93 mg/kg (Semillon), the Mn contents of the grape seeds ranged from 2.08 (Ada Karası) to 11.59 mg/kg (Cinsaut). The Cu contents of the seeds ranged between 8.62 (Narince) and 15.28 mg/kg (Chardonnay). According to Fazlo *et al.* (1982), the mean values of the Na, K and Ca contents of grape seeds were 4 660, 124 000 and 271 000 mg/kg respectively. Grape seeds can be used as a source of edible vegetable oil. It is concluded that the seeds as a by-product of the grape-processing industries in Turkey could be benefited for mainly edible oil and the other functional components. Some differences were observed in the concentrations of minerals when compared with the values in the literature. The differences in results could probably be due to differences in genetic factor, variety and cultural factors during the maturation of grape seeds.

TABLE 3
Fatty acid composition of grape seed oils (%).

Fatty acids	Papaz Karası	Cabernet	Sauvignon	Gamay	Cinsaut	Lavallé	Alfons	Sangiovese	Ada Karası	Semillon	Sauvignon blanc	Chardonnay	Narince
Palmitoleic	0.38 ± 0.03*c	0.28 ± 0.05c	0.55 ± 0.11b	0.21 ± 0.03d	0.34 ± 0.05c	1.30 ± 0.17a	1.13 ± 0.09a	0.38 ± 0.03c	0.27 ± 0.01c	0.56 ± 0.012b	0.35 ± 0.07c		
Heptadenoic	0.08 ± 0.01b**	0.05 ± 0.01c	nd	0.03 ± 0.01c	nd	0.11 ± 0.01a	0.08 ± 0.01b	0.06 ± 0.01c	0.09 ± 0.01b	0.11 ± 0.01a	0.04 ± 0.01c		
Oleic acid	20.35 ± 0.67b**	13.35 ± 0.37c	23.48 ± 1.13b	13.42 ± 0.48c	17.60 ± 0.38c	25.85 ± 0.42a	26.30 ± 1.13a	14.66 ± 0.79d	14.29 ± 0.94d	17.97 ± 0.68c	14.80 ± 0.64d		
Eicosenoic acid	0.34 ± 0.01	nd	0.18 ± 0.03	nd	nd	0.58 ± 0.11	0.33 ± 0.05	nd	nd	nd	nd	nd	
C20:1	0.12 ± 0.01	0.15 ± 0.03	0.11 ± 0.01	0.11 ± 0.01	0.16 ± 0.03	0.29 ± 0.05	0.15 ± 0.01	0.13 ± 0.01	0.15 ± 0.03	0.14 ± 0.03	0.09 ± 0.01		
ΣMUFA	21.27 ± 0.98b	13.83 ± 1.13c	24.32 ± 0.78a	13.77 ± 0.58d	18.10 ± 0.49b	28.53 ± 0.78a	27.99 ± 0.71a	15.23 ± 0.56c	14.80 ± 0.89c	18.78 ± 0.87b	15.28 ± 0.39c		
C16:1 <i>trans</i>	0.17 ± 0.3	0.11 ± 0.01	nd	nd									
ΣTrans	0.17 ± 0.03	0.11 ± 0.01	nd	nd									
Linoleic	58.40 ± 1.67b	71.76 ± 1.38a	58.35 ± 1.27	72.91 ± 2.11a	69.12 ± 1.89b	47.34 ± 0.87c	47.84 ± 0.76c	71.42 ± 2.21a	70.72 ± 2.19a	67.69 ± 1.67b	71.59 ± 1.79a		
Linolenic	0.06 ± 0.01e	0.27 ± 0.03b	0.18 ± 0.03c	0.29 ± 0.05a	0.26 ± 0.03b	0.14 ± 0.01d	0.13 ± 0.01d	0.25 ± 0.07b	0.33 ± 0.05a	0.18 ± 0.01c	0.3 ± 0.07a		
ΣPUFA	58.46	72.03	58.53	73.20	69.38	47.48	47.97	71.67	71.05	67.87	71.89		
ΣUFA	79.73	85.86	82.85	86.97	87.48	76.01	75.96	86.90	85.85	86.65	87.17		
PUFA/SFA	2.90	5.13	3.41	5.61	5.54	1.97	1.99	5.47	5.02	5.08	5.60		
Capric	nd	nd	nd	nd	nd	0.46 ± 0.03	0.14 ± 0.01	nd	nd	nd	nd	nd	
Lauric	nd	nd	nd	0.04 ± 0.01	0.03 ± 0.01	nd	nd	0.04 ± 0.01	0.03 ± 0.01	0.08 ± 0.01	0.05 ± 0.01		
Myristic	0.13 ± 0.01	0.08 ± 0.01	0.11 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.24 ± 0.03	0.17 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.03	0.05 ± 0.01		
Palmitic	13.93 ± 0.27	8.91 ± 0.47	11.91 ± 0.51	7.15 ± 0.14	8.60 ± 0.17	16.06 ± 1.21	18.24 ± 1.23	8.37 ± 0.48	8.80 ± 0.37	9.18 ± 0.59	9.69 ± 0.61		
Margaric	0.23 ± 0.03	0.18 ± 0.03	0.15 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.17 ± 0.03	0.17 ± 0.03	0.11 ± 0.01	0.08 ± 0.01		
Stearic	5.50 ± 0.11	4.22 ± 0.17	4.56 ± 0.19	5.13 ± 0.21	3.27 ± 0.31	6.55 ± 0.42	4.71 ± 0.53	4.11 ± 0.49	4.62 ± 0.71	3.25 ± 0.28	2.43 ± 0.42		
Arachidic	0.31 ± 0.42	0.64 ± 0.28	0.42 ± 0.17	0.57 ± 0.31	0.44 ± 0.09	0.41 ± 0.08	0.51 ± 0.11	0.33 ± 0.03	0.45 ± 0.07	0.63 ± 0.09	0.53 ± 0.11		
ΣSFA	20.10	14.03	17.15	13.03	12.52	23.99	24.04	13.10	14.15	13.35	12.83		

* mean ± standard deviation; ** means in the same row with the same letters are not significantly different ($p < 0.05$)

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; nd, not detected; SFA: saturated fatty acids

TABLE 4
Mineral contents of grape seeds (mg/kg)

Variety	Macro and Micro Elements				
	P	K	Ca	Mg	S
Alfons Lavallé	2819.70 ± 139.95*a	5 514.82 ± 248.27b	6 469.68 ± 145.97b	2 073.90 ± 98.24a	1 259.28 ± 65.19c
Ada Karası	2369.81 ± 175.16d**	3 118.23 ± 158.42c	8 036.76 ± 251.04a	1 249.18 ± 71.45b	1 034.62 ± 2.76d
Sauvignon blanc	2467.59 ± 126.53c	4 691.39 ± 286.21c	5 703.37 ± 167.53c	1 450.11 ± 71.24b	1 161.09 ± 60.28c
Sangiovese	2624.86 ± 226.28b	4 535.41 ± 482.03c	6 961.80 ± 551.52b	1 446.84 ± 120.17b	1 284.27 ± 113.86c
Papaz Karası	2277.65 ± 50.71d	4 225.64 ± 81.97c	5 411.08 ± 222.39c	1 397.00 ± 34.75b	1 164.64 ± 30.82c
Narince	3209.13 ± 338.00a	7 798.37 ± 524.62b	6 282.62 ± 438.61b	1 421.41 ± 175.71b	1 163.19 ± 105.95c
Gamay	2754.93 ± 232.41b	9 492.60 ± 552.01a	6 132.39 ± 637.18b	1 390.66 ± 141.03b	1 232.66 ± 83.24c
Semillon	2766.89 ± 45.63b	2 792.62 ± 170.33d	6 560.52 ± 119.61b	1 315.17 ± 40.56b	5 232.18 ± 126.73a
Cinsaut	2444.18 ± 357.81c	4 680.35 ± 469.38c	5 807.15 ± 372.94c	1 469.66 ± 271.39b	1 125.37 ± 134.30c
Chardonnay	3232.42 ± 348.94a	6 911.97 ± 763.60b	5 115.58 ± 538.96c	1 721.41 ± 175.67a	1 526.00 ± 160.54b
Cabernet Sauvignon	2823.77 ± 143.67b	4 347.80 ± 231.58c	5 363.30 ± 118.02c	1 451.34 ± 63.29b	1 363.97 ± 68.44c

* mean ± standard deviation; ** means in the same row with the same letters are not significantly different (p < 0.05)

TABLE 4 (CONTINUED)

Variety	Macro and Micro Elements				
	Fe	Zn	Mn	B	Cu
Alfons Lavallé	48.87 ± 2.17c	8.40 ± 1.25c	8.88 ± 2.22b	20.89 ± 1.80a	12.32 ± 0.48b
Ada Karası	42.79 ± 4.46d	11.71 ± 0.95b	2.08 ± 0.75d	14.30 ± 0.82b	10.88 ± 0.59c
Sauvignon blanc	49.26 ± 2.54c	10.41 ± 4.66b	4.11 ± 0.39c	15.57 ± 0.96b	10.93 ± 0.40c
Sangiovese	73.82 ± 26.35a	10.89 ± 1.61b	8.96 ± 1.32b	19.34 ± 1.33a	13.35 ± 0.73b
Papaz Karası	69.90 ± 22.12a	8.79 ± 0.14c	9.36 ± 1.14a	16.39 ± 1.87	13.89 ± 0.87b
Narince	29.96 ± 6.19d	8.27 ± 1.95c	10.50 ± 2.55a	18.03 ± 2.21a	8.62 ± 0.68
Gamay	50.65 ± 3.67c	11.28 ± 1.62b	9.29 ± 1.70a	18.53 ± 1.62a	15.15 ± 0.63a
Semillon	51.18 ± 3.87c	15.93 ± 0.71a	10.89 ± 0.20a	13.00 ± 0.91b	11.60 ± 0.28c
Cinsaut	51.53 ± 5.06c	10.84 ± 1.54b	11.59 ± 1.71	9.39 ± 1.85c	10.07 ± 0.96c
Chardonnay	56.91 ± 8.31b	11.21 ± 2.41b	8.96 ± 1.32b	14.75 ± 1.80b	15.28 ± 1.45a
Cabernet Sauvignon	64.75 ± 3.04a	14.02 ± 4.40a	7.76 ± 0.96b	12.86 ± 1.30c	12.90 ± 1.23b

* mean ± standard deviation; ** means in the same row with the same letters are not significantly different (p < 0.05)

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