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Yield and Composition of Grape Seed Oils Extracted by Supercritical Carbon Dioxide and Petroleum Ether: Varietal Effects

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Grape seed has a well-known potential for production of oil as a byproduct of winemaking and is currently produced as a specialty oil byproduct of wine manufacture. Seed oils from eight varieties of grapes crushed for wine production in British Columbia were extracted by supercritical carbon dioxide (SCE) and petroleum ether (PE). Oil yields by SCE ranged from 5.85 ± 0.33 to 13.6 ± 0.46% (w/w), whereas PE yields ranged from 6.64 ± 0.16 to 11.17 ± 0.05% (± is standard deviation). The oils contained α-, β-, and γ-tocopherols and α- and γ-tocotrienols, with γ-tocotrienol being most important quantitatively. In both SCE- and PE-extracted oils, phytosterols were a prominent feature of the unsaponifiable fraction, with β-sitosterol quantitatively most important with both extractants. Total phytosterol extraction was higher with SCE than with PE in seven of eight variety extractions. Fatty acid composition of oils from all varieties tested, and from both extraction methods, indicated linoleic acid as the major component ranging from 67.56 to 73.23% of the fatty acids present, in agreement with literature reports.

KEYWORDS: Grape seed oil; phytosterols; tocopherols; supercritical carbon dioxide

INTRODUCTION

Grape seed has a well-known potential for production of oil as a byproduct of winemaking (*1*) and is currently produced as a specialty oil byproduct of wine manufacture. Grapes are approximately 25% (w/w) dry pomace, of which about 38% (w/w) is seed (*2*). The seed may contain 10−15% oil (*1*, *2*). Rice (*2*) reports red grape seed to contain 14.34 ± 1.94% oil, whereas white grape seed contains 14.72 ± 1.62% oil. In 2001, British Columbia wineries crushed 14137 short tons of grapes of various varieties (*3*). A straightforward calculation indicates that the seed oil byproduct from wine manufacture in 2001 would have been ∼201 tons of oil, worth about $CDN 1.6−2.1 million (retail) as a specialty salad or cooking oil. The oil is very high in linoleic acid, has a pleasant, neutral taste, and has a high level of natural vitamin E, which provides for considerable oxidative stability. All of these properties contribute to grape seed oil's reputation as a good anticholesteremic, dietetic oil (*4*), which reduces low-density lipoprotein (LDL) levels and raises high-density lipoprotein (HDL) levels, providing the anticholesterol effect and protecting against heart problems.

Recently, interest has grown in various specialty oils that provide increased levels of nutrients over conventionally processed oils and are produced free of solvent residues. Supercritical carbon dioxide is the usual solvent of choice for production of these oils because of its complete dissipation on exposure to atmospheric pressure (*5*). As an extractant, supercritical CO_2 is nontoxic, nonflammable, noncorrosive, cheap, and available in large quantities at high purity in contrast to other extractants such as hexane, which is commonly used in conventional oil processing and which provides only some of these benefits. Extraction of grape seed oil with supercritical CO_2 depends on extraction pressure, temperature, particle size, particle moisture, and solvent flow rate in the apparatus used (*6*, *7*). For grape seed extraction, 40 °C and 20 MPa (200 bar) were considered to be efficient extraction conditions, although increasing the pressures and temperatures to 40 MPa and 60 °C, respctively, increased extraction rates slowly. Reduction of seed moisture content below ∼2.5% and of particle sizes to 0.35 mm by milling seed was desirable for maximum yield. In addition to this, carbon dioxide flow rates of 1.5−2.0 L/min (at STP) were recommended through the 40 g sample placed in the 75 mL sample chamber. Under these conditions, yields of supercritical CO_2 extracted oil were ∼92% of the yield obtained by hexane extraction (*6*, *7*). This difference may be attributed to the lack of phospholipids and other components in the supercritical CO_2 extracted oil removing the requirement for alkali refining and degumming of hexane-extracted oils.

The study demonstrates supercritical CO_2 extraction of grape seed oils and provides estimates of the composition of the oils available from grape seeds remaining after wine production in the Okanagan Valley of British Columbia. Eight grape varieties processed in 2001 were examined.

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MATERIALS AND METHODS

Grape pomace was obtained during the fall crush of 2001 from Calona Wines Ltd. of Kelowna, BC, Canada, and was freeze-dried for storage and later processing. Seeds were isolated by blending (Waring blender) the freeze-dried pomace at a rate determined experimentally to reduce the soft pomace to powder while preserving the hard seed virtually undamaged. The blended pomace was placed in a sieve stack consisting of no. 5, 7, 10, 35, and 60 sieves. The seeds were retained by the no. 5 and 7 (4.0 and 2.79 mm) sieves, whereas the remainder of the material was designated hulls and fines and was discarded. The seeds were cleaned further in an air classifier, and a small amount of light material (0.6−3.2% w/w, depending on variety) was removed prior to grinding of the seed in a Wily mill to pass a 2 mm screen. Particle sizes of the ground seed were 1.25 mm or smaller as determined by sieving experiments.

Supercritical carbon dioxide extraction was carried out with a Thar Designs SCFE071301 fluid extractor. Ground seed (176 g), accurately weighed, was placed in a 500 mL extraction chamber in approximately the geometric center. The void volume at either end of the chamber was filled with glass beads and glass wool to stabilize the extraction bed. Extraction with supercritical carbon dioxide was carried out over 6 h, at 37 MPa and 65 °C, at a flow rate of 60 g/min. Extracted oil was collected into a 50 mL tube treated with butylated hydroxytoluene (BHT) dissolved in ethanol such that 1.7 mg of BHT was left after evaporation of the ethanol. The oil samples containing BHT were flushed with nitrogen, tightly capped, and stored at −70 °C for later analysis. Oil extraction was monitored over time by weighing the receiving tube during the extraction. Conventional oil determinations were done according to an AOAC method (*8*) (method 920.39) in a Goldfisch apparatus using petroleum ether (30−60 °C) as the extraction solvent on an accurately weighed 3−4 g sample.

Tocopherols. Tocopherols were measured according to a modification of the method of Oomah et al. (*4*) using a Waters HPLC system equipped with Waters Millennium software version 3.05.01 and a fluorescence detector (McPherson SF-749 spectrofluorometer, Acton, MA). Separation occurred on a normal phase column (4.6 × 150 mm, Primesphere 5 silica, 5 μm, Phenomenex, Torrance, CA) with a guard column (4.6 × 30 mm) of the same material. Isocratic elution occurred with heptane/2-propanol/2,2-dimethoxypropane (1000:3:1 v/v/v) at ∼21 °C, flowing at 1.6 mL/min. Detector excitation was set at 297 nm and emission detection at 325 nm. Quantitation was based on an external standard method for α- and δ-tocopherols (Sigma Chemical Co., St. Louis, MO) and for β- and γ-tocopherols (Matreya, Pleasant Gap, PA). Prior to analysis, oils were diluted with heptane to obtain a concentration near 70 mg/mL and filtered through a PTFE Acrodisc filter (0.2 μm, Gelman Science Inc., Ann Arbor, MI). Injection volume was 20 μL.

Fatty Acid Determination. Oil (30 mg) was weighed into screw-capped vials and, sequentially, 1 mL of tetrahydrofuran at ambient temperature (∼21 °C) and 1 mL of methanolic 1 M KOH were added and vortexed briefly. After 1 min of standing, 1 mL of boron trifluoride (14% in methanol, Pierce Chemical, Rockford, IL) was added and mixed thoroughly. The mixture was heated for 15 min at 100 °C and then cooled, and 0.5 mL of saturated NaCl was added. Heptadecanoic acid (1 mL, 1 mg/mL, in isooctane) was added followed by 1 mL of isooctane. After thorough mixing, the upper layer was used directly for gas chromatography (GC). Chromatography was performed using a Supelco SP-2560 fused silica capillary [100 m × 0.25 mm i.d., 0.20 μm film thickness (Supelco, Bellefonte, PA)] column in an Agilent 6890 GC (Agilent Technolgies Inc., Wilmington, DE) equipped with a flame ionization detector. Samples (1 μL) were injected using a model 7683 autoinjector and a split−splitless injector with a split ratio of 10:1. The oven program consisted of an initial temperature of 140 °C for 5 min, followed by a temperature ramp to 240 °C at 4 °C/min. The temperature was held at 240 °C for 30 min. Injector and detector temperatures were 260 °C, and carrier gas (helium) was used in constant pressure mode (average linear flow rate = 22 cm/s). The instrument was controlled and data collected and quantitated with an Agilent ChemStation (version G2070AA). Analysis was done in duplicate.

Phytosterols. These compounds were measured essentially as described by Beveridge et al. (*15*). Reference phytosterols dihydrocholesterol (internal standard), lupeol, sitostanol, β-sitosterol, and squalene were from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada), and β-amyrin and erythrodiol were from Extrasynthese S.A. (Genay, France). A plant sterol mixture containing cholesterol, brassicasterol, campesterol, stigmasterol, and β-sitosterol were from BDH Inc. (Toronto, ON, Canada). The unsaponifiable fraction was prepared by adding to 1 g of oil 1 mL of 0.1% (w/v) dihydrocholesterol (3β-cholestanol; internal standard) in *tert*-butyl methyl ether. KOH (20 mL, 1 M) in methanol was added and stirred overnight at ambient temperature (∼21 °C). This solution was diluted with 40 mL of distilled water and extracted (three times) with 30 mL of *tert*-butyl methyl ether. The combined organic extract was washed with 15 mL of 0.5 M aqueous KOH, followed by repeated 30 mL distilled water washes until the pH of the washes was the same as that of the original water, followed by one wash with 15 mL of saturated NaCl. The solvents were removed by rotary vacuum evaporation at 30 °C, and the residue was left under continuous vacuum overnight to remove solvent traces. Preparations were prepared in duplicate and stored at −20 °C in the dark, until further analysis.

Table 1. Yields of Oil from Eight Varieties of Grape Seed Determined by Supercritical Carbon Dioxide (SCE) and Petroleum Ether (30–60 °C) (PE)[a]

grape variety	yield by SCE (%, w/w)	yield by PE (%, w/w)
Barbera	6.14 ± 0.25	6.71 ± 0.07
Gamay	5.85 ± 0.33	6.64 ± 0.16
Malbec	9.36 ± 0.99	10.78 ± 0.12
Pinot Noir	10.7 ± 0.81	9.83 ± 0.05
Merlot	10.5 ± 0.95	10.75 ± 0.12
Cabernet Franc	10.7 ± 0.47	10.29 ± 0.18
Syrah	10.8 ± 1.26	10.10 ± 0.10
Cabernet Sauvignon	13.6 ± 0.46	11.17 ± 0.05

[a] ± values are standard deviation; $n = 2$ (SCE); $n = 3$ (PE). Yield of SCE is after 6 h of extraction.

TMS ether derivatives were prepared by adding 300 μL of Tri-Sil (Pierce Chemicals, Rockford, IL) reagent to 15−100 mg of unsaponifiable in glass-stoppered tubes and mixing. The tubes were heated at 60 °C for 60 min, and then excess reagent and solvent were removed under a nitrogen stream at ambient temperature. The residue was dissolved in 2 mL of hexane for quantitative gas chromatography with flame ionization detection (FID) or 0.1−0.3 mL followed by gas chromatography−mass spectrometry (GC-MS) for identification purposes. Analysis of the TMS ether derivatives was done using a DB-5 fused silica capillary column (60 m × 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA) in an Agilent 6890 GC (Agilent Technologies inc., Wilmington, DE) equipped with a flame ionization detector. Samples (1 μL) were injected using a 7683 autoinjector and a split−splitless injector with a split ratio of 10:1. Oven temperature was isothermal at 275 °C, injector and detector temperatures were 280 and 300 °C, respectively, and helium carrier gas was used in constant pressure mode at 24 psi (average linear velocity = 28 cm/s). The machine was controlled and components were analyzed using an Agilent ChemStation (version G2070AA). All samples were run in duplicate. GC-MS was performed with a Hewlett-Packard 5890A gas chromatograph equipped with a split−splitless injector, a 5970 mass selective detector (MSD) with split interface, and a 7673A injector. The GC to MSD transfer line was set at 300 °C, and the MSD parameters were as follows: scan mode, 50−600 amu; threshold, 400; sample rate, 1.1 scans/s; ionizing voltage, 70 eV; EM voltage, 2000 V. The GC and MSD were controlled and MS data collected by an HP-Chemstation as described for the FID system above. Mass spectral identification was done by using the Wily MS database and/or comparing the spectra to literature reports.

RESULTS AND DISCUSSION

The cumulative extraction of oil from ground grape seed over time is shown in **Figure 1**. All varieties showed a region of rapid oil extraction followed by a region of slow to very slow

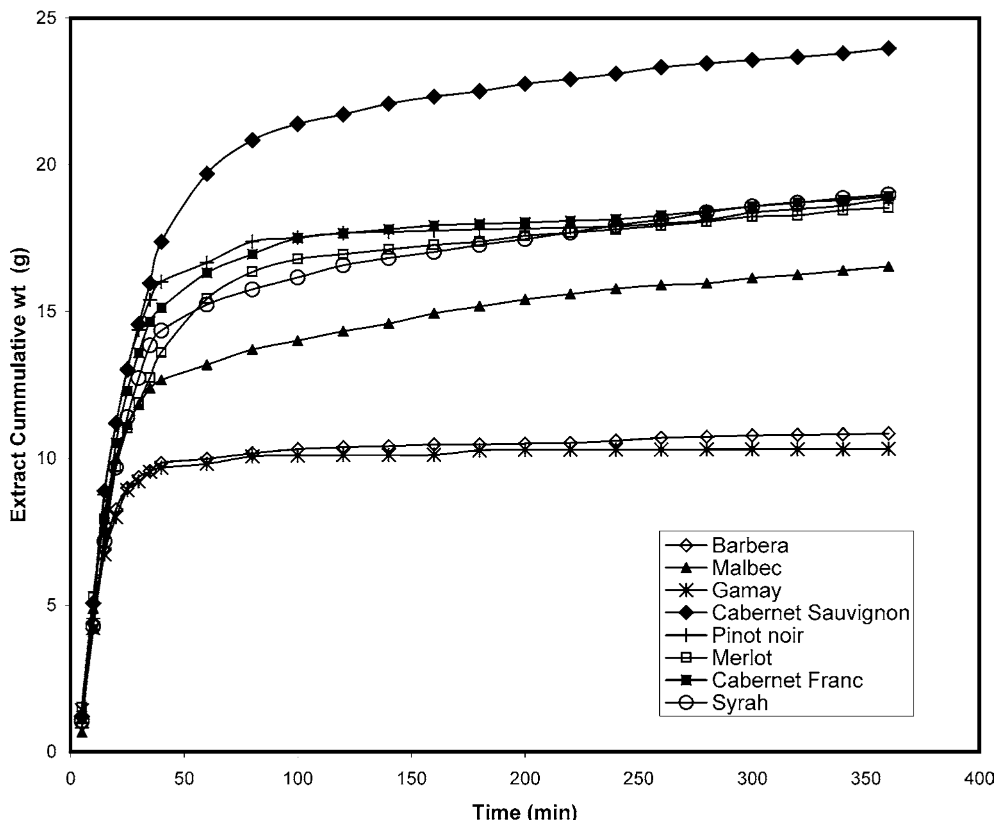

Figure 1. Cumulative extraction of oil from 176 g of ground grape seed of eight varieties by supercritical carbon dioxide at 65 °C, 37 MPa, and carbon dioxide flow rate of 60 g/min. Each point represents the average of two runs.

Table 2. Tochopherols and Tocotrienols Determined by HPLC in Grape Seed Oil Extracted with Supercritical Carbon Dioxide (SCE) or Petroleum Ether (30–60 °C) (PE)[a]

grape variety	α	β	γ	α_{T3}	γ_{T3}
Extracted with SCE (Milligrams per 100 g of Oil)					
Barbera	14.4 ± 0.79	7.20 ± 1.62	7.92 ± 0.82	16.3 ± 0.94	24.3 ± 1.68
Malbec	12.6 ± 0.35	6.36 ± 1.03	7.42 ± 0.36	17.1 ± 0.67	21.7 ± 0.39
Gamay	30.9 ± 0.90	11.9 ± 2.73	14.1 ± 0.47	19.6 ± 0.52	24.7 ± 1.36
Cabernet Sauvignon	8.70 ± 0.79	4.89 ± 0.77	4.44 ± 1.91	14.6 ± 1.22	37.5 ± 2.89
Pinot Noir	11.7 ± 0.13	5.02 ± 0.34	5.00 ± 0.27	14.7 ± 0.31	35.0 ± 0.46
Merlot	7.67 ± 0.36	4.83 ± 1.19	2.82 ± 0.36	21.2 ± 0.12	33.2 ± 1.56
Cabernet Franc	16.1 ± 0.55	6.49 ± 0.77	6.74 ± 0.40	10.2 ± 0.19	33.5 ± 1.09
Syrah	14.1 ± 0.14	4.00 ± 0.48	9.87 ± 0.18	13.8 ± 0.67	28.5 ± 1.46
Extracted with PE (Milligrams per 100 g of Oil)					
Barbera	11.6 ± 0.45	6.72 ± 1.26	6.64 ± 1.15	12.6 ± 0.29	23.0 ± 1.49
Malbec	9.70 ± 0.63	4.64 ± 1.48	5.56 ± 0.26	11.9 ± 0.59	22.0 ± 0.35
Gamay	27.2 ± 0.84	15.3 ± 0.26	13.2 ± 0.54	18.7 ± 0.31	29.0 ± 1.87
Cabernet Sauvignon	3.58 ± 0.54	2.15 ± 0.29	2.59 ± 0.31	10.4 ± 0.79	38.3 ± 1.55
Pinot Noir	10.5 ± 0.24	5.62 ± 0.42	5.08 ± 0.18	14.6 ± 0.20	35.9 ± 1.11
Merlot	7.76 ± 0.06	4.09 ± 0.26	2.06 ± 0.14	22.8 ± 0.20	32.8 ± 0.65
Cabernet Franc	14.4 ± 0.26	8.41 ± 0.36	6.77 ± 0.27	11.3 ± 0.30	37.6 ± 0.59
Syrah	12.0 ± 0.15	4.42 ± 0.16	10.0 ± 0.22	12.2 ± 0.26	27.6 ± 0.94

[a] ± values are standard deviations ($n = 4$). Symbols with a sub T3 denote tocotrienols.

additional oil yield. This is in agreement with the results reported by several authors for grape and other oil seeds (*5*, *7*, *9*). It has been suggested that this represents a two-mechanism extraction process with rapid extraction of surface and shallow subsurface oil followed by diffusion-controlled extraction of the more deeply embedded oil (*10*). The eight varieties of grape seed analyzed here fall into three general categories depending on the yield of oil from the variety (**Figure 1**; **Table 1**). The group of seeds that yielded the least oil [Gamay (5.85%) and Barbera (6.14%)] were extracted very rapidly within ~60 min, and very little additional oil was extracted with increased extraction time. The grape varieties representing intermediate levels of oil content [Malbec (9.36%), Syrah (10.8%), Merlot (10.5%), Cabernet Franc (10.7%), and Pinot Noir (10.7%)] were also nearly completely extracted within 60 min, whereas Cabernet Sauvgnon (13.6%) ground seed might be advantageously extracted for an additional 60 min. The values (in parentheses above; **Table 1**) behind each variety name are the yield (percent, w/w) after 360 min of extraction.

Tocopherols are particularly important in grape seed oils as these oils are a commonly available source of vitamin E activity even after the oil is refined (*11*), a process that removes these compounds from conventionally produced vegetable oils. The levels obtained in the eight varietal grape seed oils studied here

Table 3. Phytosterols (Milligrams per 100 g of Oil) in Grape Seed Oil Extracted with Supercritical Carbon Dioxide[a]

	grape variety							
phytosterol	Barbera	Malbec	Gamay	Cabernet Sauvignon	Pinot Noir	Merlot	Cabernet Franc	Syrah
squalene	31.8	18.6	80.1	14.9	30.0	8.8	37.0	28.3
campesterol	58.0	45.2	103.4	46.0	57.0	40.6	46.8	49.4
stigmasterol	54.9	54.9	106.4	47.6	51.1	43.0	56.7	44.6
β-sitosterol	549.5	373.0	1120.3	338.5	442.3	245.5	408.5	396.8
β-amyrin + sitostanol	30.7	18.3	79.7	18.1	21.7	9.8	21.2	34.0
Δ-5-avenastenol	16.2	12.7	20.7	13.4	13.7	9.5	14.5	12.3
lupeol	4.89	5.32	11.7	3.47	10.2	3.52	3.36	6.21
Δ-7-sitosterol	34.2	15.1	40.4	9.0	13.6	8.1	22.1	15.6
Δ-avenasterol	2.9	2.8	1.7	1.4	1.4	2.6	2.1	1.7
24-methylenecycloartanol	17.2	11.6	13.9	8.2	12.0	8.3	17.0	28.8
erythrodiol + citrostadienol (tr)	20.2	9.8	97.2	19.2	18.8	8.3	13.6	7.5
unknown	137.8	12.4	186.1	44.1	70.2	36.8	80.6	38.2
total	958.5	579.7	1861.7	563.9	742.0	410.3	723.0	597.0

[a] Values represent the average of duplicate analyses.

Table 4. Phytosterols (Milligrams per 100 g of Oil) in Grape Seed Oil Extracted with Petroleum Ether[a]

	grape variety							
phytosterol	Barbera	Malbec	Gamay	Cabernet Sauvignon	Pinot Noir	Merlot	Cabernet Franc	Syrah
squalene	26.3	22.7	34.6	5.7	13.0	7.7	18.2	20.4
campesterol	52.8	44.8	81.9	36.5	49.8	36.6	42.2	42.3
stigmasterol	50.1	43.2	85.5	36.5	45.5	40.0	50.0	39.6
β-sitosterol	494.5	368.7	682.3	196.9	346.1	207.6	310.1	326.4
sitosterol	27.8	17.8	43.6	8.8	18.1	8.1	15.8	13.3
Δ-5-avenasterol	16.7	44.9	20.1	11.9	10.0	9.1	13.0	16.4
lupeol	5.37	2.64	5.26	2.89	5.08	3.32	3.21	6.43
Δ-7-sitosterol	33.7	18.1	19.8	3.9	9.9	7.0	15.7	12.8
Δ-avenasterol	2.6	1.6	1.4	1.1	1.1	2.8	1.7	3.4
24-methylenecyclostadienol	19.0	nd	8.9	9.3	12.0	8.0	15.4	26.0
erythrodiol + citrostadienol	18.8	nd	45.4	3.0	11.9	7.5	9.2	10.4
unknown	57.2	18.7	77.8	nd	18.0	2.4	18.6	11.3
total	805.0	602.8	1106.5	316.5	540.6	340.3	513.2	528.8

[a] Values were obtained from a single run and so represent estimates.

Table 5. Fatty Acid Composition (Percent of Total) of Grape Seed Oil Extracted with Supercritical Carbon Dioxide[a]

	grape variety							
fatty acid	Barbera	Malbec	Gamay	Cabernet Sauvignon	Pinot Noir	Merlot	Cabernet Franc	Syrah
C14:0	0.10	0.06	0.12	0.06	0.09	0.05	0.11	0.07
C16:0	6.58	6.28	7.82	6.82	7.61	7.07	8.26	7.30
C16:1	0.12	0.12	0.14	0.06	0.15	0.08	0.11	0.13
C18:0	4.38	4.65	3.60	4.92	4.15	3.95	5.22	4.40
C18:1	16.40	18.47	14.38	12.71	14.74	13.13	13.02	15.10
C18:2	69.44	67.56	68.94	72.57	70.13	73.23	70.28	70.15
C18:3 (α)	0.66	0.54	1.12	0.48	0.65	0.44	0.65	0.67
C20:0	0.18	0.22	0.26	0.21	0.21	0.17	0.26	0.20
total	97.86	97.89	96.38	97.83	97.72	98.14	97.89	98.03

[a] Reported as the average of duplicate analyses.

are given in **Table 2** for both supercritical carbon dioxide and petroleum ether extracted oils. The levels obtained for α-, β-, and γ-tocopherols may be compared to literature values of 1.6−3.8 (α), 0−8.9 (β), 0−7.3 (γ), and 0−0.4 (δ) mg/100 g (*4*) and 0.54−5.59 (α), 0.21−2.31 (β), 0.84−3.31 (γ), and 0−6.97 (δ) mg/100 g (*4*) for the α-, β-, γ-, and δ-forms respectively, remembering that the δ-forms are not reported in **Table 2**. Comparable tocotrienol levels were 1.8−10.7 (α), 11.5−20.5 (γ), and 0−0.3 (δ) mg/g (*12*) and 0.67−15.7 (α), 15.23−28.48 (γ), and 0 (δ) mg/g for the α-, γ-, and δ-forms, respectively. The values obtained here (**Table 2**) for all of the tocopherols are very much higher than literature values, but the tocotrienol values are in general agreement with the literature. Tocopherols and tocotrienols are stated to be highly sensitive to light and air (oxygen) and are acknowledged to be subject to losses from these effects during preparation (*11*). In the present case, we attempted to mitigate these effects by extracting the oils into vessels treated with 1.7 mg of dry BHT, nitrogen flushing, and storage at very low temperatures (−70 °C) prior to analysis. These actions would be expected to preserve both the tocopherols and tocotrienols in the oils, and extraction with supercritical carbon dioxide would enhance this preservation

Table 6. Fatty Acid Composition (Percent of Total) of Grape Seed Oil Extracted with Petroleum Ether[a]

	grape variety							
fatty acid	Barbera	Malbec	Gamay	Cabernet Sauvignon	Pinot Noir	Merlot	Cabernet Franc	Syrah
C14:0	0.10	0.03	0.07	0.06	0.07	0.05	0.11	0.08
C16:0	6.82	6.35	7.15	6.84	7.50	6.90	8.62	7.93
C16:1	0.14	0.13	0.11	0.06	0.14	0.07	0.12	0.19
C18:0	4.46	4.73	3.64	5.03	4.09	3.95	5.26	4.31
C18:1	16.62	18.95	14.56	12.63	15.00	12.84	13.72	16.61
C18:2	68.57	66.76	70.78	72.77	70.00	73.61	69.06	68.00
C18:3 (α)	0.68	0.54	0.79	0.53	0.62	0.50	0.62	0.69
C20:0	0.19	0.24	0.20	0.20	0.19	0.17	0.27	0.20
total	97.58	97.76	97.31	98.13	97.60	98.08	97.78	98.02

[a] Reported as average of duplicate analyses.

further because oxygen is excluded during the process. If tocopherols and tocotrienols are easily degraded during preparation and the literature adequately reflects this degradation, then there must be a difference in the susceptibilities to degradation between tocopherols and tocotrienols. Apparently only the tocopherols needed protection from degradation during isolation and analysis. Assay of both δ-tocopherol and δ-tocotrienol proved to be uncertain by this method because the peak eluting at the expected position was not always present, even in duplicate analyses. Why this difficulty arose is unknown, but the method is subject to peak migration to longer elution times over multiple injections, a condition not unknown for this assay (*13*), and the levels of these δ-compounds are very low and variable (*4*, *12*) in grape seed oil. This combination of factors probably contributed to difficulties in the identification and quantification with certainty. As a result, values for the δ-tocopherols and δ-tocotrienols are not reported. However, it is probable that small quantities of both are present in the oils.

Phytosterols in plant oils are of increasing interest because of their role in the control of cholesterol levels and, by implication, reduction of arteriosclerosis. In the popular literature this activity in grape seed oil is attributed to the high levels of linoleic acid present in grape seed oil; however, phytosterols are well-known to contribute anti-arteriosclerotic activity and may make a major contribution in grape seed oil. The results of a phytosterol analysis of grape seed oil obtained by supercritical carbon dioxide and petroleum ether extractions are shown in **Tables 3** and **4**. Reports of phytosterols in grape seed oil are scarce, but Tiscornia and Bertini (*14*) report the presence of cholesterol (0.2−0.4%), brassicasterol (0−trace), campesterol (10.2−10.5%), stigmasterol (11.8−12.2%), β-sitosterol (74.2−75.3%), and Δ^7-stigmasterol (2.2−3%). Firestone (*12*) reports cholesterol (0−0.5%), brassicasterol (0−0.2%), campesterol (9−14%), stigmasterol (9−17%), β-sitosterol (present), Δ^5-avenasterol (1−3%), Δ^7-stigmasterol (1−3%), Δ^7-avenasterol (0−1%), and other sterols such as sitostanol and $\Delta^{5,24}$-stigmasterol. Total sterols are reported as 580 mg/100 g of oil. Campesterol, stigmasterol, β-sitosterol, and Δ^5-avenasterol are common findings and β-sitosterol is, quantitatively, the most important sterol. Also, the total sterol value of 580 mg/100 g of oil in the literature is within the range of values reported here. The unknown of **Tables 3** and **4** contains oleanolic acid as one component, representing perhaps 40% of the total as indicated by concurrence of retention time and MS fragmentation of authentic standard. From a comparison of the values of **Tables 3** and **4** it appears that supercritical carbon dioxide is a more effective extractant for the phytosterols compared to petroleum ether. In almost every case, the level of total phytosterols or the level of individual phytosterol is higher in the supercritical carbon dioxide extracted samples. One exception is the total phytosterol value for the variety Malbec, which is lower in **Table 3** than in **Table 4**. The variety Gamay is high in phytosterol levels compared to the other varieties tested, being remarkably high when extracted with supercritical carbon dioxide (**Table 3**). Other than Gamay, the levels of phytosterols are lower than those in ginseng oil (total = 798−974 mg/100 g) (*15*) and much lower than that found in sea buckthorn seed oil (total = 1369−1441 mg/100 g) (*16*).

Fatty acid analysis (**Tables 5** and **6**) of the grape seed oils derived by both petroleum ether and supercritical carbon dioxide gave results completely in agreement with consensus value ranges published in ref *12*. All varieties tested provided a typical linoleic-rich oil (*1*) by both extraction methods, and there was little variation between the oils extracted by the different extraction methods. The values obtained here are also in agreement with values for native American hybrid varieties except that the American varieties had lower stearic acid levels (*17*).

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