

## Characterization of Canadian Black Currant (*Ribes nigrum* L.) Seed Oils and Residues

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The seeds from five black currant (*Ribes nigrum* L.) cultivars grown in western Canada were evaluated for their oil content, fatty acid and triacylglycerol (TAG) composition, and tocopherol and phytosterol profiles and contents. Moreover, polyphenolic compounds and antioxidant activity in the seed extracts remaining after oil extraction were determined. Oil contents of black currant seeds ranged from 27 to 33%. The  $\gamma$ -linolenic acid content varied significantly among the cultivars (from 11% for Ben Conan to 17% for Ben Tirran). Among the 44 TAGs identified, LL $\alpha$ Ln,  $\alpha$ LnL $\gamma$ Ln, and PL $\gamma$ Ln (where L = linoleoyl,  $\alpha$ Ln =  $\alpha$ -linolenoyl,  $\gamma$ Ln =  $\gamma$ -linolenoyl, and P = palmitoyl) were the predominant ones. Black currant seed oil was a good source of tocopherols (1143 mg/100 g of oil on average) and phytosterols (6453 mg/100 g of oil on average). Quercetin-3-glucoside and *p*-coumaric acid were the main phenolic components in the seed residues. The high concentration of flavonols and phenolic acids was correlated with a high antioxidant activity of seed residue (average ABTS value of 1.5 mM/100 g and DPPH value of 1.2 mM/100 g). The data obtained from this study indicate that Canadian black currant seed oil is a good source of essential fatty acids, tocopherols, and phytosterols. Extraction of phenolic antioxidants from the seed residues even allows the recovery of additional valuable components from the byproduct of fruit processing.

**KEYWORDS:** Black currants; seed oils; fatty acids; triacylglycerols; tocopherols; phytosterols; polyphenols

### INTRODUCTION

Black currant (*Ribes nigrum* L.) berries are widely cultivated for use in beverages, jellies, and jams and are reputed to be excellent for health because of their high contents of antioxidants (1). The berries are rich in anthocyanins, which are responsible for their dark color (2). They also contain considerable amounts of other flavonoids and phenolic acids (3).

During black currant juice production, large amounts of press residues (pomace) including seeds are obtained. The oil from black currant seeds contains various fatty acids of nutritional significance, namely  $\gamma$ -linolenic [18:3(n-6)],  $\alpha$ -linolenic [18:3(n-3)], and stearidonic acids [18:4(n-3)]. In humans,  $\alpha$ -linolenic acid is the immediate precursor of stearidonic acid, which after elongation and desaturation gives rise to eicosapentaenoic acid [20:5(n-3)], the precursor of eicosanoids that show anti-inflammatory and antithrombotic activities (4). Stearidonic acid has shown anticancer, antithrombotic, and anti-inflammatory activities (5).  $\gamma$ -Linolenic acid is a metabolite of linoleic acid [18:2(n-6)] and an intermediate in the bioconversion of linoleic to arachidonic acid [20:4(n-6)] in humans. Hypertension (6), diabetes (7), and cancer (8) are some of the conditions shown to be attenuated by supplementation of the diet with  $\gamma$ -linolenic.

The content and composition of fruit seed oils are affected by the geography and local climate (9). Whereas the lipid profile of black currant seed oils from different regions in Europe has already been reported, no information is available on the lipid composition in Canadian black currant seed oils.

The seeds are byproducts of fruit processing, and the seed residue is the primary byproduct of seed oil production. Recent studies showed that berry seed residues may contain significant levels of antioxidants (10). Furthermore, extracts obtained from these residues demonstrated chelating capacities and antiproliferative activities.

These data suggest the potential of fruit seed residues as a source of valuable food ingredients that can improve human diets while at the same time the profitability of fruit production and processing can be enhanced. Additional research is required to investigate other fruit seed residues for their contents of health beneficial compounds to promote their utilization.

Recently it was shown that the profile of phenolic compounds in black currant seed residues, that is, phenolic acids, flavonols, and anthocyanins, is identical to that present in the berries (11). However, no information was provided on their concentration and antioxidant activity. The present study was conducted to investigate the seed oil extracted from Canadian black currant cultivars for its fatty acid, triacylglycerol (TAG), tocopherol, and phytosterol composition. Moreover, the seed residues were investigated for their polyphenol contents and antioxidant

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activities. Multivariable data analysis based on oil and residue composition was performed to evaluate the differences in the profile among cultivars. The data obtained from this study could be used to promote the utilization of black currant seed oil and press residues in food products for improving human health.

## MATERIALS AND METHODS

**Chemicals.** Citric acid, sodium acetate, sodium hydroxide, sodium chloride, potassium chloride, potassium hydroxide, boron trifluoride, hexane, methanol (ACS grade), acetonitrile (HPLC grade), 2-propanol (HPLC grade), pyrogallol, cyclohexane, and anhydrous sodium sulfate were purchased from Fisher Scientific (Ottawa, ON, Canada); pyridine was obtained from Pierce (Rockford, IL); 5 $\alpha$ -cholestane was from Sigma-Aldrich (St. Louis, MO); *N,O*-bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane were from Alltech (Guelph, ON, Canada). Folin–Ciocalteu reagent, gallic acid, potassium persulfate, benzylmercaptan, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma (St. Louis, MO); cyanidin-3-glucoside and cyanidin-3-rutinoside were from Indofine (Hillsborough, NJ); chlorogenic acid, rutin, quercetin-3-glucoside, quercetin-3-galactoside, kaempferol-3-glucoside, kaempferol-3-rutinoside, and myricetin-3-glucoside were purchased from ChromaDex (Santa Ana, CA). Fatty acid methyl ester standards were purchased from Nu-Chek Prep Inc. (Elysian, MN). The plant sterol mixture and tocopherol standards were obtained from Matreya LLC (Pleasant Gap, PA), and triacylglycerol standards were purchased from Sigma-Aldrich.

**Berry Material.** Ripe black currant berries from five cultivars (Ben Alder, Ben Conan, Ben Tirran, Ben Sarek, and Ben Nevis) were harvested in July 2006 on Don Smith commercial plantation (Elnora, AB, Canada). The seeds were removed from the berries, washed with tap water, and air-dried at room temperature.

**Oil Content.** The oil content of the berry seeds was determined according to the method of Troeng (12). Seeds were ground in a commercial grinder. Two and a half grams of accurately weighed and well-mixed sample was transferred into a "Swedish Tube" containing ball bearings [manufactured according to specification of the American Oil Chemists' Society (AOCS)], and 35 mL of petroleum ether (boiling range 36–60 °C as specified by AOCS) was added. The samples were then shaken on a reciprocating shaker (Eberbach Corp., Ann Arbor, MI) for 20–30 min and subsequently filtered under vacuum. The washings of petroleum ether were collected in a tared flask, and the solvent was removed under vacuum using a rotary evaporator. The oil content was expressed on a dry seed weight. Data are reported as a mean value  $\pm$  standard deviation (SD) of two samples of each cultivar, analyzed individually in triplicate.

**Moisture Content.** The moisture content of black currant seed was determined according to AOCS Method Ba 2a-38 (13). For this purpose, 3 g of seed was dried for 2 h at 130 °C. Data are reported as a mean value  $\pm$  standard deviation (SD) of two samples of each cultivar, analyzed individually in triplicate.

**Fatty Acid Composition.** The fatty acid composition of seed oils was determined by gas–liquid chromatography of fatty acid methyl esters according to AOCS Official Methods Ce 2-66, Ce 1e-91, and Ce 1b-89 (14). Oil (0.25 g) was hydrolyzed with 6 mL of 0.5 N NaOH in methanol solution. When the oil was completely dissolved, indicating complete hydrolysis, 6 mL of boron trifluoride (14% in methanol solution) was added and the reaction mixture refluxed under heat for approximately 5 min. Subsequently, 10 mL of hexane was added, and the mixture was cooled to ambient temperature. Twenty milliliters of 15% NaCl solution was then added to the flask. The clear hexane solution was transferred to an autosampler vial using a Pasteur pipet. Analyses were performed on an Agilent 6890 gas chromatograph equipped with an Agilent 7683 autosampler, a flame ionization detector, and a 30 m  $\times$  0.32 mm i.d., 0.25  $\mu$ m, DB225 capillary column (Agilent Technologies, Mississauga, ON, Canada). The temperature program was as follows: 200 °C for 9 min, raised at 4 °C/min to 220 °C, and held at 220 °C for 7 min. The detector and injector temperatures were 280 and 250 °C, respectively. The carrier gas was helium at a constant pressure of 15 psi. The samples (1  $\mu$ L) were injected at a split rate of 1:100. Data are reported as a mean value  $\pm$  SD of two samples of each cultivar, analyzed individually in triplicate.

**Tocopherol and Phytosterol Composition.** The tocopherol and phytosterol composition of the seed oils was determined by gas–liquid chromatography according to the method of Slover et al. (15) with modifications. For this purpose, 0.1 g of oil was saponified with 0.5 mL of saturated aqueous KOH in the presence of 8 mL of 3% ethanolic pyrogallol for 8 min in a water bath at 80 °C. Eight milliliters of water was added, and unsaponifiables were extracted three times with 10 mL of cyclohexane. After removal of the solvent, tocopherols and phytosterols were derivatized with pyridine, *N,O*-bis(trimethylsilyl)trifluoroacetamide, and trimethylchlorosilane. TMS derivatives were analyzed using an Agilent 6890 gas chromatograph (Agilent Technologies) equipped with a 30 m  $\times$  0.32 mm, i.d., 0.25  $\mu$ m, HP-1 capillary column. The temperature program was as follows: 240 °C for 20 min, raised at 5 °C/min to 260 °C, and held at 260 °C for 26 min. The detector and injector temperatures were 300 and 280 °C, respectively. The carrier gas was helium at a constant pressure of 15 psi. The samples (2  $\mu$ L) were injected at a split rate of 1:50. 5 $\alpha$ -Cholestane was used as an internal standard at a concentration of 0.2 mg/mL. The response factors (tocopherols versus 5 $\alpha$ -cholestane and phytosterols versus 5 $\alpha$ -cholestane) used for calculation were 1.08 and 1.0, respectively. Data are reported as a mean value  $\pm$  SD of two samples of each cultivar, analyzed individually in triplicate.

**Characterization of Triacylglycerols by HPLC-APCI-MS.** The characterization of triacylglycerols in black currant seed oil by HPLC-APCI-MS<sup>n</sup> was performed according to the method of Lisa and Holčapek (16) with modifications. The seed oils were dissolved in an acetonitrile/2-propanol (1:1, v/v) mixture yielding 3% (w/v) solutions. The chromatographic system consisted of an 1100 series Agilent Technologies LC-MSD system equipped with a diode array detector (DAD) coupled to a mass spectrometer (quadrupole analyzer) fitted with an atmospheric pressure chemical ionization (APCI) interface (Agilent Technologies). The separation was performed on two 250 mm  $\times$  4.6 mm, i.d., 4  $\mu$ m, RP C12 columns (Synergi Max-RP 80A, Phenomenex, Torrance, CA) using gradient elution with acetonitrile (A) and 2-propanol (B). The elution system was as follows: 0 min, 100% A; 129 min, 31% A; 132 min, 100% A. MS parameters were as follows: vaporizer temperature, 400 °C; drying gas temperature, 350 °C; desolvation gas flow (N<sub>2</sub>), 3.0 L/min; nebulizer gas pressure (N<sub>2</sub>), 60 psi. The instrument was operated in positive ion mode scanning from *m/z* 100 to 1200 at a scan rate of 2.0 s/cycle.

**Extraction of Polyphenols from Defatted Seed Residues.** Seed residues were extracted three times at ambient temperature with 50% acetone (3  $\times$  10 mL solvent/g of flour) according to the method of Parry et al. (10) with modification. Between extractions, the samples were centrifuged at 2000g for 10 min. The combined supernatants were evaporated at 40 °C under vacuum. The residue was dissolved in 0.1% aqueous formic acid and the solution analyzed for total polyphenols, total anthocyanins, and antioxidant activities. For HPLC analysis, the solution was applied to a column of Amberlite XAD-16 nonionic polymeric adsorbent (Rohm and Haas, Philadelphia, PA). After washing with 0.1% aqueous formic acid, the polyphenol fraction was collected by elution with methanol containing 0.1% formic acid. The eluate was evaporated to dryness. The residue was dissolved in 0.1% aqueous formic acid. Extractions were performed in triplicate.

**Determination of Total Polyphenols.** Total polyphenol contents of the extracts were determined using the Folin–Ciocalteu colorimetric method as described by Singleton et al. (17) with modifications. One milliliter of seed residue extract and 1 mL of Folin–Ciocalteu reagent were pipetted into a 100 mL volumetric flask. After 3 min, 10 mL of 20% aqueous solution of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) was added, and the flask was made up to 100 mL with distilled water. The absorbance at 765 nm was measured after 1 h, and the results were expressed as milligrams of gallic acid equivalents per 100 g of residue. Data are reported as a mean value  $\pm$  SD of six measurements.

**Determination of the Antioxidant Activity.** The antioxidant activity of the seed residue extracts was determined using the Trolox equivalent antioxidant capacity (TEAC) with ABTS and DPPH radicals. The ABTS assay was carried out according to the method of Re et al. (18), whereas the DPPH assay was performed according to the procedure described by Yen and Chen (19). TEAC results were expressed as millimoles of Trolox equivalents (TE) per 100 g of black currant seed residue. Data are reported as mean value  $\pm$  SD of six measurements.

**Table 1.** Oil Content and Fatty Acid Composition (Percent) of Black Currant Seed Oil<sup>a</sup>

	Ben Tirran	Ben Sarek	Ben Alder	Ben Conan	Ben Nevis
oil content <sup>b</sup>	27.1 ± 5.4c	30.1 ± 2.5b	29.7 ± 7.1b	28.5 ± 6.6c	32.8 ± 9.2a
12:0	0.0	T <sup>c</sup>	0.0	T	0.0
14:0	0.1	0.1	0.1	0.1	T
16:0	6.6 ± 1.1a	6.1 ± 0.8b	6.0 ± 0.6b	5.8 ± 0.3c	5.9 ± 0.4c
16:1(n-7)	0.3	0.2	0.2	0.2	0.2
18:0	1.7 ± 0.2a	1.8 ± 0.1a	1.7 ± 0.2a	1.5 ± 0.3a	1.9 ± 0.3a
18:1(n-9)	11.0 ± 0.2b	12.3 ± 1.3a	11.2 ± 0.4b	11.8 ± 1.4a	11.8 ± 1.4a
18:2(n-6)	43.9 ± 3.2b	45.3 ± 3.6b	44.4 ± 8.7b	46.5 ± 3.7a	47.5 ± 6.9a
18:3(n-6)	16.7 ± 6.7a	15.2 ± 2.8a	14.7 ± 5.4a	10.9 ± 8.7b	12.7 ± 6.1b
18:3(n-3)	14.5 ± 3.1c	14.1 ± 2.1c	16.5 ± 4.4b	18.1 ± 1.6a	14.9 ± 3.7c
18:4(n-3)	3.5 ± 0.2a	3.0 ± 0.6b	3.5 ± 0.4a	3.1 ± 0.2b	2.9 ± 0.2b
20:0	0.2	0.2	0.2	0.2	0.2
20:1(n-9)	1.1 ± 0.2a	1.1 ± 0.1a	1.0 ± 0.2a	1.3 ± 0.1a	1.4 ± 0.3a
20:2(n-6)	0.3	0.3	0.3	0.3	0.3
22:0	0.1	0.1	0.1	0.1	0.1
22:1(n-9)	T	T	T	T	T
24:0	T	0.1	T	T	0.1
others	0.2	0.1	0.2	0.2	0.2
SFA	8.7 ± 0.9a	8.4 ± 1.1b	8.1 ± 1.4b	7.6 ± 2.0c	8.2 ± 2.1b
MUFA	12.3 ± 3.2b	13.7 ± 1.8a	12.4 ± 1.6b	13.4 ± 2.3a	13.3 ± 2.2a
PUFA	78.9 ± 8.9a	77.9 ± 11.4b	79.3 ± 13.2a	78.9 ± 10.1a	78.3 ± 13.4b
n-6/n-3	3.4 ± 0.2b	3.6 ± 0.4a	3.0 ± 0.1c	2.7 ± 0.2c	3.4 ± 0.3b

<sup>a</sup> Values are mean ± SD of two samples of each cultivar, analyzed individually in triplicate. Different letters within each row represent significant difference ( $p < 0.05$ ). <sup>b</sup> Percent of dry seed weight. <sup>c</sup> T, trace.

**Characterization of Polyphenols by HPLC-ESI-MS.** HPLC-ESI-MS/MS analyses were performed using an 1100 series Agilent Technologies LC-MSD system equipped with a DAD coupled to a mass spectrometer (quadrupole analyzer) fitted with an electrospray ionization (ESI) interface (Agilent Technologies). Polyphenols were analyzed using an RP C18 column 250 × 4.6 mm (Grace Vydac, Hesperia, CA). The compounds were separated with gradient elution using 4.5% aqueous formic acid (A) and 80% acetonitrile in solution A (B) as eluents. The elution system was as follows: 0–7 min, 15% B; 7–15 min, 20% B; 15–16 min, 100% B, at a flow rate of 1 mL/min. MS parameters were as follows: capillary voltage, 4000 V; drying gas temperature, 350 °C; desolvation gas flow (N<sub>2</sub>), 12 L/min; nebulizer gas pressure (N<sub>2</sub>), 60 psi; smart parameter setting (SPS); compound stability, 70%. The instrument was operated in both positive and negative ion modes scanning from  $m/z$  100 to 1500 at a scan rate of 2.0 s/cycle.

**Statistical Analysis.** Analysis of variance (one-way ANOVA) was performed using Statgraphic software (StatPoint, Inc., Sainte-Fey, QC, Canada). A probability value of  $p \leq 0.05$  was considered to denote a statistically significant difference. Multivariable data analysis was performed using Unscrambler 9.8 software (CAMO Software Inc., Woodbridge, NJ).

## RESULTS AND DISCUSSION

**Oil Content.** The seed oil contents of the five Canadian black currant cultivars are summarized in **Table 1**. The yields (27.1–32.8%) varied significantly ( $P \leq 0.05$ ) within the cultivars analyzed, with Ben Nevis showing the highest and Ben Tirran the lowest oil contents. Thus, Canadian black currants seeds had higher oil contents than Finnish (15.9%) (20) and Bulgarian (22.0%) black currant seeds (21) and were also superior in this respect to 10 black currant cultivars grown in western Europe (17.2–22.3% oil) (22).

**Fatty Acid Composition.** The fatty acid compositions are presented in **Table 1**. Black currant seed oil contained <9% of saturated fatty acids (SFA), which is in agreement with data presented for Finnish black currant seed oil (20). Palmitic and stearic acids were the main components among SFAs. Total contents of monounsaturated fatty acids (MUFA), which mainly consisted of oleic acid, ranged from 12.3% in Ben Tirran to 13.7% in Ben Sarek. The oil was rich in polyunsaturated fatty

acids (PUFA), ranging from 77.9% (Ben Sarek) to 79.3% (Ben Alder). Thus, Canadian black currant seed oils contained PUFA concentrations similar to those reported in wild Finnish black currant seed oil (80% PUFAs) (20) and higher concentrations than reported for Bulgarian black currant seed oil (71% PUFAs) (21). Among the PUFAs, linoleic acid was the predominant fatty acid (45.5% on average).

Black currant seed oil is known to be a good source of  $\gamma$ -linolenic and stearidonic acids, which are essential fatty acids that have important roles as precursors of other long-chain polyunsaturated fatty acids and hormones in humans (5, 23). In Canadian black currant seed oil, significant variations in the content of  $\gamma$ -linolenic acid were observed, with the cultivar Ben Conan containing the lowest level (10.9%) and Ben Tirran the highest concentration (16.7%). These contents are comparable to those found in 10 black currant cultivars grown in western Europe (11.9–15.8%) (22). Genotypic variation in fatty acid composition in seed oils extracted from black currants grown in Scotland was reported recently (24). The  $\gamma$ -linolenic acid content varied from 11 to 19% of the total fatty acids, but three genotypes had higher values of 22–24%, contents previously not reported for black currant seed oil. Stearidonic acid contents in Canadian black currant seed oil ranged from 2.9% for Ben Nevis to 3.5% for Ben Tirran and Ben Alder. These contents are similar to the values reported for Finnish and Scottish berries (20, 24).

Canadian black currant seed oils also had very low ratios of n-6 to n-3 fatty acids (3.2 on average), suggesting that inclusion of these seed oils in the diet may contribute to reduce the ratio of n-6 to n-3 fatty acids. There is increasing evidence that lowering the n-6 to n-3 ratio may be beneficial in reducing the risk of cancer and cardiovascular disease and enhancing bone health (25, 26).

**Triacylglycerol Composition.** The fatty acid composition can be used to evaluate the stability and nutritional quality of fats and oils. However, to understand their physical and functional properties, the determination of the types and amounts of TAG species present in the oil is also essential. The TAG identification and composition of black currant seed oil is presented in **Tables 2** and **3**. Overall, the TAG profiles of the five cultivars of Canadian black currant seed oil were similar; however, significant changes

**Table 2.** Molecular Mass and Fragmentation Ions Obtained for TAG Identified in Black Currant Seed Oil by HPLC-DAD-APCI-MS

TAG <sup>a</sup>	R <sub>time</sub> (min)	[M + H] <sup>+</sup>	[M + H - R <sub>i</sub> COOH] <sup>+</sup>	[R <sub>i</sub> COOH] <sup>+</sup> , [R <sub>i</sub> COOH + 58] <sup>+</sup>
StαLnSt	49.2	869.8	591.5 (100) <sup>b</sup>	259.3 (45)
StγLnSt	50.4	869.8	591.5 (100)	259.3 (45)
αLnαLnSt	53.8	871.8	593.5 (80), 595.5 (60)	261.1 (60), 259.2 (35)
γLnαLnSt	54.8	871.8	593.5 (80), 595.5 (60)	261.1 (60), 259.2 (30)
γLnγLnSt	56.1	871.7	593.5 (80), 599.5 (60)	335.3 (50)
αLnαLnαLn	58.4	873.8	595.5 (90)	261.1 (60)
αLnαLnγLn	59.5	873.8	595.5 (90)	261.1 (60)
αLnLnSt	59.8	873.9	593.5 (70), 597.5 (55)	335.2 (40)
γLnαLnγLn	61.0	873.8	595.5 (100)	261.1 (55)
γLnLnSt	61.0	873.8	593.5 (70), 597.5 (55)	335.2 (60)
γLnγLnγLn	62.3	873.8	595.5 (100)	261.1 (65)
αLnαLn	64.4	875.7	595.5 (80), 597.5 (50)	
αLnLnγLn	65.7	875.7	595.5 (80), 597.5 (50)	337.3 (30)
LLSt	67.0	875.8	597.5 (80), 599.5 (45)	337.2 (40)
γLnLnγLn	67.1	875.7	597.5 (90), 595.5 (65)	337.3 (60)
StαLnO	67.9	876.7	597.5 (90), 593.5 (60), 599.1 (40)	339.3 (55)
StαLnP	68.5	850.8	593.5 (100), 571.5 (70)	313.2 (55), 259.2 (30)
LLαLn	70.9	877.7	599.5 (95), 597.5 (60)	
LLγLn	72.1	877.7	599.5 (95), 597.5 (60)	337.3 (30)
αLnαLnO	72.4	877.7	599.5 (100), 595.5 (55)	
αLnαLnP	73.4	851.7	573.5 (80), 595.5 (60)	255.2 (65)
γLnαLnO	73.8	877.7	599.5 (100), 595.5 (60)	
OLSt	74.3	878.8	595.5 (80), 597.5 (60), 601.5 (40)	339.3 (40)
PLSt	74.6	851.8	573.5 (80), 595.5 (40)	313.3 (100)
LLL	76.8	879.7	599.5 (100)	337.2 (80), 263.2 (10)
OLαLn	77.4	879.7	601.5 (100), 597.5 (65), 599.4 (40)	261.3 (40)
OOST	77.6	879.8	597.5 (80), 603.5 (30)	259.3 (70), 339.5 (30)
PLαLn	78.2	854.0	575.5 (100), 597.5 (45), 573.5 (30)	335.1 (45), 261.1 (40)
OLγLn	78.8	879.7	601.5 (100), 597.5 (50), 599.4 (40)	339.3 (70)
PLγLn	79.6	854.0	575.5 (80), 597.5 (50), 573.5 (40)	313.3 (80), 335.3 (60)
αLnαLnS	80.9	879.8	601.6 (100), 595.5 (55)	341.3 (50)
unknown	82.6	907.8	627.6 (90), 625.5 (75), 599.5 (15)	
LLO	83.7	881.8	599.5 (100), 601.6 (55)	339.3 (70), 263.2 (20)
LLP	84.5	855.7	599.5 (100), 575.4 (65)	313.3 (35)
αLnOO	85.7	881.8	599.5 (80), 603.5 (55)	
αLnLS	86.0	881.8	597.5 (80), 603.5 (50), 601.5 (45)	341.3 (50)
αLnOP	86.4	855.8	573.5 (100), 599.5 (65), 577.5 (40)	265.3 (70), 239.3 (30)
LLG	89.2	909.8	599.5 (100), 629.5 (55)	367.3 (80), 337.3 (30), 339.3 (25)
OLO	90.2	883.7	601.5 (80), 603.5 (60)	265.2 (100), 339.3 (40)
OLP	91.1	857.7	601.5 (100), 577.5 (75), 575.5 (70)	313.2 (70)
SOαLn	92.0	883.7	605.6 (80), 599.5 (45)	341.2 (100), 265.3 (30)
GLO	95.6	911.9	631.6 (70), 629.6 (55), 601.6 (30)	293.3 (100), 265.3 (50)
OOO	96.6	885.8	603.6 (90)	265.3 (65)
OOP	97.3	859.7	577.5 (85), 603.5 (70)	265.3 (60), 239.3 (45)

<sup>a</sup> Abbreviations: αLn, α-linolenate; γLn, γ-linolenate; L, linoleate; O, oleate; S, stearate; St, stearidonate; P, palmitate; G, gadoleate. <sup>b</sup> Relative abundance of the fragment ion.

( $p \leq 0.05$ ) in the TAG concentration of black currant cultivars were found. HPLC-APCI-MS analysis allowed the identification of 44 TAG species, which contained mainly α-linolenic [18:3(n-3)], linoleic [18:2(n-6)], γ-linolenic [18:3(n-6)], oleic [18:1(n-9)], and palmitic (16:0) acids (Figure 1). The predominant components were detected at  $m/z$  877.7, 875.7, and 854.0 and were identified as dilinoleoyl-α-linolenoylglycerol (LLαLn), α-linoleoyl-linoleoyl-γ-linolenoylglycerol (αLnLnγLn), and palmitoyl-linoleoyl-γ-linolenoylglycerol (PLγLn), respectively (Table 2).

The positional distribution of the fatty acids within the TAGs was determined by the ratio of fragment ions  $[M + H - R_i\text{COOH}]^+$ . Because the neutral loss of fatty acids from the *sn*-2 position is less favored in comparison to *sn*-1 and *sn*-3 positions, it provides a fragment ion with lower relative abundance than statistically expected. In agreement with previously published data (16), the *sn*-2 position in black currant seed oil was preferentially occupied by unsaturated fatty acids, mainly linoleic acid.

A complete separation of all TAG species was not achieved under the chromatographic conditions applied. Therefore, some

of them were summarized as shown in Table 3. Twenty-two TAGs contained at least one of two essential fatty acids typical of black currant oil (stearidonic and γ-linolenic acids), which are mainly located in the *sn*-1 and *sn*-3 positions. Among the five cultivars investigated, Ben Tirran contained the highest amounts of TAGs with γ-linolenic acid (51.6%) and Ben Conan the lowest (41.2%). Ben Tirran also had the highest amount of TAGs containing stearidonic acid (25.5%), whereas Ben Conan and Ben Nevis contained levels of around 19%. Only small amounts of saturated fatty acids were found in the seed oils (8.2% in average) (Table 1). TAG species containing two or three saturated fatty acids were not detected, and those TAGs with one saturated fatty acid moiety contained palmitic or stearic acids (Tables 2 and 3).

The TAG composition of black currant seed oils from the Czech Republic was reported recently (16). The authors identified 77 TAGs, with LLO, LLL, LLLn, OLLn, LLP, and PLγLn being the main TAGs. The qualitative composition of TAGs in Canadian black currant seed oil was quite similar, but proportions were different. The main difference found between Czech and

**Table 3.** Triacylglycerol Composition of Black Currant Seed Oil<sup>a</sup>

TAG	Ben Tirran	Ben Sarek	Ben Alder	Ben Conan	Ben Nevis
StαLnSt	0.3	0.3	0.2	0.2	0.2
StγLnSt	0.1	0.1	0.1	0.1	0.1
αLnαLnSt	0.8	0.7	0.6	0.8	0.6
γLnαLnSt	1.5 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
γLnγLnSt	0.4	0.2	0.2	0.2	0.2
αLnαLnαLn	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.5 ± 0.2	0.7 ± 0.1
αLnαLnγLn	4.4 ± 0.2a	3.7 ± 0.1b	3.7 ± 0.3b	4.5 ± 0.4a	3.5 ± 0.2c
αLnLnSt					
γLnαLnγLn	4.1 ± 0.2a	3.3 ± 0.3b	3.1 ± 0.2b	2.4 ± 0.3c	2.5 ± 0.3c
γLnLnSt					
γLnγLnγLn	0.6	0.5	0.3	0.4	0.2
αLnLnαLn	4.3 ± 0.2b	4.2 ± 0.3b	4.6 ± 0.2b	7.0 ± 0.3a	4.9 ± 0.1b
αLnLnγLn	10.6 ± 1.1a	9.7 ± 0.9b	9.8 ± 1.0b	9.6 ± 0.8b	9.9 ± 0.9b
LLSt	5.3 ± 0.3a	4.8 ± 0.2b	3.8 ± 0.4c	3.1 ± 0.4d	3.7 ± 0.3c
γLnLnγLn					
StαLnO	1.3 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	1.0 ± 0.2	1.0 ± 0.1
StαLnP					
LLαLn	9.5 ± 0.2c	9.8 ± 0.3c	12.2 ± 0.4b	13.6 ± 0.7a	11.9 ± 0.9b
LLγLn	11.6 ± 1.0a	11.9 ± 0.8a	10.1 ± 0.7b	9.5 ± 0.6b	11.0 ± 1.0a
αLnαLnO					
αLnαLnP	3.5 ± 0.3a	3.7 ± 0.2a	3.1 ± 0.2b	3.0 ± 0.4b	3.2 ± 0.3a
γLnαLnO					
OLSt	1.4 ± 0.1	1.1 ± 0.2	1.3 ± 0.2	1.5 ± 0.1	1.1 ± 0.1
PLSt	2.1 ± 0.3	1.8 ± 0.3	1.5 ± 0.1	1.0 ± 0.2	1.3 ± 0.1
LLL	10.1 ± 1.9d	11.2 ± 1.0c	13.3 ± 1.1b	14.0 ± 1.3a	13.0 ± 1.4b
OLαLn					
OOST	2.6 ± 0.5b	2.7 ± 0.3b	3.1 ± 0.1a	2.5 ± 0.3b	3.0 ± 0.2a
PLαLn					
OLγLn	4.0 ± 0.2b	4.7 ± 0.2a	4.1 ± 0.5b	3.5 ± 0.4c	4.2 ± 0.2b
PLγLn	5.6 ± 0.3a	5.3 ± 0.2a	4.8 ± 0.3b	3.9 ± 0.2c	4.8 ± 0.3b
αLnαLnS	0.6	0.6	0.4	0.3	0.5
unknown	0.3	0.1	0.2	0.3	0.2
LLO	3.0 ± 0.2b	4.0 ± 0.3a	4.5 ± 0.2a	4.0 ± 0.2a	4.7 ± 0.2a
LLP	5.0 ± 0.3b	5.3 ± 0.3b	6.0 ± 0.3a	5.7 ± 0.3a	5.9 ± 0.3a
αLnOO	1.7 ± 0.1b	2.0 ± 0.2a	1.9 ± 0.1a	1.3 ± 0.2c	1.8 ± 0.3b
αLnLS					
αLnOP	0.8	0.7	0.6	0.5	0.6
LLG	0.2	0.3	0.6	0.6	0.6
OLO	1.5 ± 0.1	2.0 ± 0.1	1.8 ± 0.2	1.6 ± 0.1	1.9 ± 0.1
OLP	1.0 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
SOαLn	0.1	0.2	0.2	0.2	0.2
GLO	0.1	0.1	0.1	0.1	0.1
OOO	0.1	0.2	0.2	0.2	0.2
OOP	0.2	0.2	0.3	0.2	0.3

<sup>a</sup> TAG composition presented as DAD mean peak area (%) recorded at 205 nm; *n* = 3. Different letters within each row represent significant difference (*p* < 0.05). For abbreviations, see footnote in Table 2.

Canadian seed oils was the concentration of LLO, and the predominant TAG present in Czech oil was found only in average concentrations in the Canadian oils. The Canadian black currant seed oils seem to have higher concentrations of TAGs containing  $\gamma$ -linolenic acid. Although this suggests that Czech black currant seed oil contains lower amounts of  $\gamma$ -linolenic acid, a direct comparison of fatty acid composition is not possible because only the TAG composition of seed oil was reported by Lísá and Holčápek (16).

**Tocopherol Composition.** The tocopherol contents of the seed oils are shown in Table 4.  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherols were found in all oils investigated, whereas  $\beta$ -tocopherol was not detected in any of the samples. Total tocopherol contents varied significantly within cultivars. The highest tocopherol content was found in the cultivar Ben Sarek (1417 mg/kg of oil) and the lowest in Ben Nevis (811.4 mg/kg of oil).  $\gamma$ -Tocopherol was the main vitamin E derivative and accounted for 68–82% of total tocopherol, followed by  $\delta$ -tocopherol (9–25%) and  $\alpha$ -tocopherol (5–9%).

Tocopherols in vegetable oils protect polyunsaturated fatty acids from oxidation.  $\alpha$ -Tocopherol has the highest vitamin E bioactivity, but only  $\delta$ -tocopherol shows the ability to detoxify nitrogen dioxide (27).

The tocopherol content of seed oils of 10 black currant cultivars from western Europe ranged between 1228 and 2458 mg/kg of oil (22). Similar to the Canadian black currant seed oil,  $\gamma$ -tocopherol was the predominant vitamin E compound (54–65%) in the European seed oils, with the concentration of  $\alpha$ -tocopherol being higher than found in the present study. Canadian black currant seed oils were higher in their tocopherol contents than Bulgarian seed oils (250 mg/kg of oil) (21).

**Phytosterol Composition.** The qualitative and quantitative composition of phytosterols in black currant seed oil is shown in Table 4. Significant differences in phytosterol concentration between the cultivars were found, with Ben Tirran and Ben Alder containing the highest amounts of phytosterols (6894 and 6797 mg/kg of oil, respectively), followed by the cultivars Ben Conan and Ben Sarek (6426 and 6362 mg/kg of oil, respectively). Ben Nevis was characterized by the lowest amount of phytosterols (5787 mg/kg of oil).  $\beta$ -Sitosterol was the predominant compound in the sterol fraction (72% in average). Other phytosterols detected at lower level included campesterol (7% on average), citrostadienol (6% on average), and  $\Delta^5$ -avenasterol (3% on average).  $\beta$ -Sitosterol is known to be the principal sterol found in many seeds and oilseeds. Studies have shown that people with a diet containing 60–130 mg/day of plant  $\beta$ -sitosterol have a lower incidence of prostate cancer (28). Phytosterols also appear to play a role in modulating immune function and inflammation by affecting the production of inflammatory cytokines (28, 29). To the best of our knowledge this is the first report about the presence of citrostadienol in black currant seed oil.

The levels of  $\beta$ -sitosterol found in the present study are in agreement with those reported for Bulgarian black currant seed oil (21). More recently, a wide range (5590–25820 mg/kg of oil) of phytosterols in black currant seed oil was reported (30).  $\beta$ -Sitosterol was the main compound in the phytosterol fraction in Bulgarian seed oil, followed by campesterol and avenasterol.

**Total Polyphenols Content and Composition in Seed Residue.** Total polyphenols of seed residue extracts ranged from 160.4 mg/100 g for Ben Alder to 230.7 mg/100 g for Ben Sarek (Table 5). These concentrations in black currant seed residues were lower than those reported for raspberry, blueberry, and cranberry seed flours (10). Also, grape seed residue contained higher levels of total phenolics (6–10 g/100 g) (31). The seeds used in the present study were rinsed with water before the oil extraction to remove the phenolic compounds originating from the flesh. This step was not performed in the other studies, and this might be the reason for the lower phenolic contents found in the Canadian black currant seed residue.

The LC-MS method used in the present study allowed the identification of the main phenolic compounds. Low quantities of four anthocyanins (3–6 mg/100 g) were detected in all samples analyzed (Table 5). These anthocyanins were previously identified in black currant seed residues (11) and black currant fruits (32). Six flavonols were identified as glucosides and rutinosides of kaempferol, quercetin, and myricetin. Their presence in black currant seed residue was also reported previously by Lu and Foo (11). Flavonols were the main phenolic group in black currant seed residue, ranging from 48 mg/100 g (Ben Conan) to 73 mg/100 g (Ben Sarek). Thus, the contents of flavonols in black currant seed residues was higher than in black currant berries (18–38 mg/100 g of fresh weight) (33). Flavonols in the berries are dominated by quercetin (34) or myricetin (35). Similarly to berries, quercetin-3-glucoside was the major flavonol in the seed

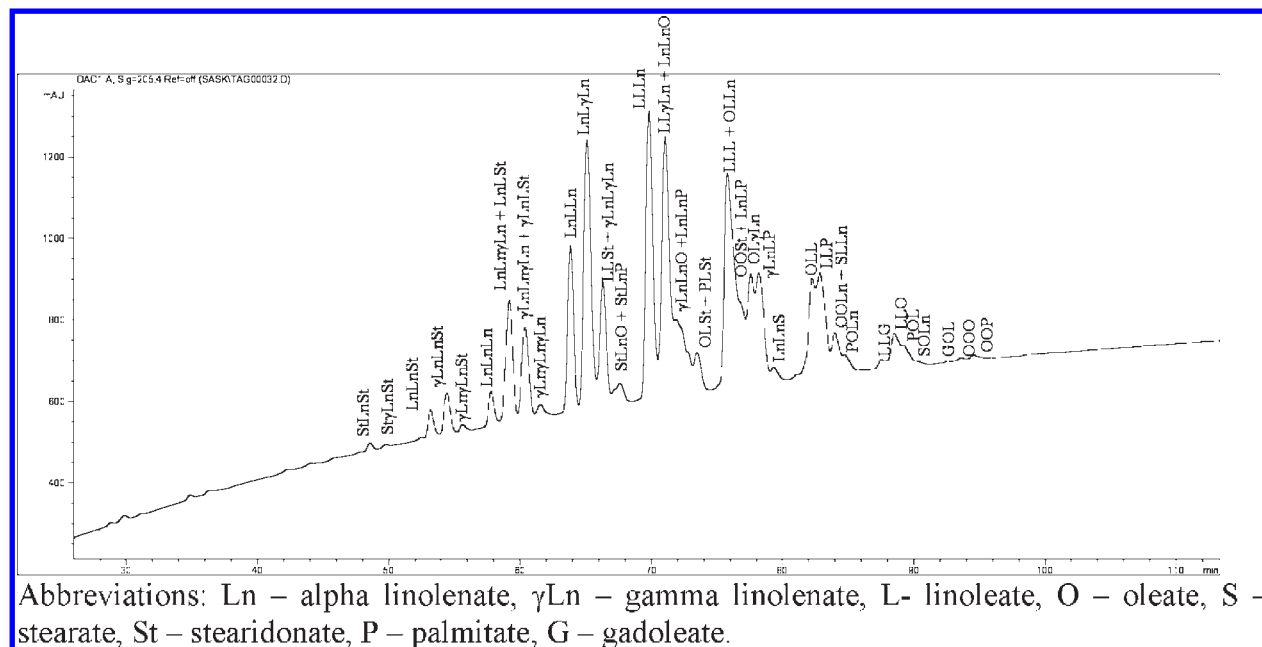


Figure 1. HPLC profile of black currant seed oil triacylglycerols (205 nm).

Table 4. Concentrations (Milligrams per 100 g of Oil) of Tocopherols and Phytosterols in Black Currant Seed Oil<sup>a</sup>

	Ben Tirran	Ben Sarek	Ben Alder	Ben Conan	Ben Nevis
$\alpha$ -tocopherol	66.8 ± 24.1c	70.8 ± 19.3b	81.3 ± 21.6b	115.9 ± 30.1a	52.3 ± 11.4c
$\gamma$ -tocopherol	130.9 ± 22.9c	205.0 ± 37.9a	158.9 ± 21.1b	119.1 ± 10.3c	206.2 ± 19.4a
$\delta$ -tocopherol	862.7 ± 44.2b	1140.9 ± 54.2a	857.5 ± 37.1b	1091.9 ± 55.5a	552.9 ± 23.4c
total tocopherols	1060.3b	1416.6a	1097.7b	1326.9a	811.4c
campesterol	504.9 ± 35.6a	504.0 ± 29.8a	434.5 ± 31.3b	511.2 ± 37.2a	403.3 ± 22.2b
stigmasterol	23.7 ± 3.6b	30.1 ± 6.2a	28.8 ± 7.1a	31.0 ± 3.6a	27.5 ± 4.1b
$\beta$ -sitosterol	5053.0 ± 68.9a	4579.2 ± 57.1b	4955.0 ± 95.2a	4691.0 ± 67.7b	3984.3 ± 82.1c
$\Delta^5$ -avenasterol	292.3 ± 21.1a	159.0 ± 19.9b	241.8 ± 20.9a	200.9 ± 17.8b	222.4 ± 31.4a
$\Delta^7$ -stigmasterol	176.3 ± 41.0a	143.1 ± 25.1b	182.4 ± 24.9a	152.9 ± 23.5b	143.6 ± 46.6b
$\Delta^7$ -avenasterol	132.6 ± 31.5a	98.21 ± 18.9c	135.1 ± 24.1a	122.0 ± 19.9a	110.2 ± 10.0b
citrostadienol	306.6 ± 23.3e	396.2 ± 25.6c	435.3 ± 45.4b	346.6 ± 36.7d	558.2 ± 41.2a
others	405.0 ± 51.2b	452.1 ± 29.7a	384.3 ± 38.3b	370.0 ± 63.2b	338.3 ± 33.5c
total sterols	6894.4a	6361.91b	6797.2a	6425.6b	5787.8c

<sup>a</sup> Values are mean ± SD of two samples of each cultivar, analyzed individually in triplicate. Different letters within each row represent significant difference ( $p < 0.05$ ).

residues, followed by myricetin-3-glucoside and kaempferol-3-glucoside (Table 5).

The seed residues contained high amounts of *p*-coumaric acid and its glucoside (44–67 mg/100 g). It is likely that *p*-coumaric acid is a hydrolysis product of its glycosides. The presence of *p*-coumaric acid and its glycosides in New Zealand black currant seed residue was reported previously (11). The percentage of *p*-coumaric acid in the phenolic fraction of the seed residues ranged between 27 and 39% and was higher than in black currant berries (24%) (34).

#### Antioxidant Activity of Black Currant Seed Residue Extracts.

The extracts of all fruit seed residues exhibited significant antioxidant capacity, with ABTS values of 1.4–1.7 TE mM/100 g and DPPH values of 1.1–1.3 TE mM/100 g (Table 5). Ben Sarek and Ben Tirran seed residues had higher antioxidant capacities among the five cultivars on a per weight basis. The antioxidant activity of Canadian black currant seed residue was comparable to the antioxidant capacity of black currant press residue (around 2.0 TE mM/100 g), which contained not only seeds but also the skins and flesh of the berries (36). These data suggest that black currant seed residues are good dietary sources for phenolic antioxidants.

**Statistical Analysis.** Table 6 shows the correlation coefficients among oil, fatty acid, tocopherol, and phytosterol concentrations

in the five cultivars of Canadian black currant. Oil and phytosterol concentrations were significantly negatively correlated, which may be explained by a dilution or concentration effect of the sterols in the oil; that is, higher oil contents lead to a reduction of the concentration of phytosterols in the oil and vice versa.  $\beta$ -Sitosterol was strongly positively correlated with  $\delta$ -tocopherol, suggesting a possible relationship between both components. A potential relationship was also found between campesterol and stearidonic acid. Stearidonic acid and  $\gamma$ -linolenic acid were significantly negatively correlated with linoleic acid. A possible explanation of this fact could be that black currant cultivars which displayed a high proportion of linoleic acid exhibited a low content of both  $\gamma$ -linolenic and stearidonic acids.  $\alpha$ -Linolenic acid was strongly positively correlated to  $\alpha$ -tocopherol, suggesting a potential relationship between these components. Moreover, strong positive correlation was found between  $\gamma$ -tocopherol and oil content. A previous study carried out on *Ribes* species (23) also found a positive correlation between oil content and  $\gamma$ -tocopherol, suggesting a strong relationship between both components.

The correlations between phenolic components and antioxidant activity of seed residues are shown in Table 7. Total polyphenol content was strongly positively correlated with

**Table 5.** Polyphenols Content (Milligrams per 100 g) and Antioxidant Activity (Millimolar per 100 g) of Black Currant Seed Residue<sup>a</sup>

	Ben Tirran	Ben Sarek	Ben Alder	Ben Conan	Ben Nevis
total polyphenols	198.8 ± 14.6b	230.7 ± 24.9a	160.4 ± 27.1bc	185.1 ± 31.5b	174.7 ± 25.1bc
delphinidin-3-glucoside	0.6 ± 0.1c	1.4 ± 0.1a	0.9 ± 0.1b	0.9 ± 0.1b	1.1 ± 0.1b
delphinidin-3-rutinoside	1.2 ± 0.1c	1.2 ± 0.2c	1.4 ± 0.2b	1.6 ± 0.1b	2.2 ± 0.3a
cyanidin-3-glucoside	0.2 ± 0.03d	1.0 ± 0.1a	0.5 ± 0.1c	0.4 ± 0.1c	0.6 ± 0.1b
cyanidin-3-rutinoside	1.0 ± 0.1c	1.9 ± 0.3a	1.4 ± 0.2b	1.5 ± 0.3b	2.2 ± 0.1a
myricetin-3-glucoside	14.1 ± 2.5b	24.0 ± 4.1a	10.5 ± 2.9c	10.4 ± 5.7c	9.1 ± 2.1c
myricetin-3-rutinoside	3.9 ± 1.1b	4.9 ± 0.8a	3.3 ± 0.5b	1.7 ± 0.3c	2.9 ± 0.2b
quercetin-3-glucoside	34.6 ± 5.7a	29.3 ± 7.7b	28.3 ± 5.3b	21.8 ± 3.6c	34.2 ± 6.4a
rutin	4.2 ± 0.8b	2.5 ± 0.3c	5.9 ± 0.3a	3.6 ± 0.5b	2.6 ± 0.4c
kaempferol-3-glucoside	7.5 ± 0.6a	6.4 ± 0.4b	6.1 ± 0.3b	4.7 ± 0.3b	7.4 ± 0.5a
kaempferol-3-rutinoside	5.9 ± 0.7a	6.8 ± 0.9a	4.9 ± 0.6b	5.4 ± 0.4b	6.4 ± 0.4a
<i>p</i> -coumaric acid	48.7 ± 8.6a	45.9 ± 6.8a	31.6 ± 3.7b	34.6 ± 7.2b	30.5 ± 8.3b
<i>p</i> -coumaroyl glycoside	17.9 ± 2.1a	15.8 ± 5.2a	16.3 ± 6.0a	14.6 ± 4.9b	13.0 ± 2.5b
total	139.8a	141.1a	111.2b	101.4b	112.2b
ABTS	1.6 ± 0.4a	1.7 ± 0.2a	1.4 ± 0.5b	1.5 ± 0.1b	1.4 ± 0.3b
DPPH	1.2 ± 0.2a	1.3 ± 0.2a	1.1 ± 0.1b	1.2 ± 0.3b	1.2 ± 0.3b

<sup>a</sup> Values are mean ± SD of two samples of each cultivar, analyzed individually in triplicate. Different letters within each row represent significant difference ( $p < 0.05$ ).

**Table 6.** Correlations among Oil Components in Five Black Currant Cultivars

	C18:2n-6	C18:3n-6	C18:3n-3	C18:4n-3	$\alpha$ -tocopherol	$\delta$ -tocopherol	$\gamma$ -tocopherol	$\beta$ -sitosterol	campesterol	$\Delta^5$ -avenasterol
oil content	0.735	-0.370	-0.178	-0.657	-0.482	-0.569	0.825	-0.895	-0.803	-0.425
C18:2n-6		-0.815	-0.226	-0.888	0.004	-0.323	0.388	-0.401	-0.912	-0.491
C18:3n-6			-0.744	0.640	-0.552	0.018	0.110	0.164	0.516	0.423
C18:3n-3				-0.007	0.871	0.225	-0.628	0.077	0.163	-0.061
C18:4n-3					0.005	-0.02	-0.545	0.119	0.856	0.783
$\alpha$ -tocopherol						0.660	-0.703	0.535	0.391	-0.228
$\delta$ -tocopherol							-0.314	0.854	0.494	-0.495
$\gamma$ -tocopherol								-0.529	-0.700	-0.505
$\beta$ -sitosterol									0.565	0.455
campesterol										-0.133

**Table 7.** Correlations among Seed Residue Components and Antioxidant Activity of Five Black Currant Cultivars<sup>a</sup>

	D-3-glc	D-3-rut	C-3-glc	C-3-rut	M-3-glc	M-3-rut	Q-3-glc	Rutin	K-3-glc	K-3-rut	CA	CA-glc	TP
TP	0.394	-0.552	0.582	0.043	0.761	0.660	0.924	-0.634	0.068	0.070	0.895	0.369	
ABTS	0.083	-0.711	0.309	-0.256	0.639	0.741	0.863	-0.415	0.251	0.253	0.759	0.521	0.932
DPPH	0.265	-0.539	0.471	-0.020	0.801	0.724	0.880	-0.616	0.274	0.276	0.728	0.512	0.974

<sup>a</sup> Abbreviations: D, delphinidin; C, cyanidin; M, malvidin; Q, quercetin; K, kaempferol; CA, *p*-coumaric acid; glc, glucoside; rut, rutinoside; TP, total polyphenols.

the main components in the phenolic fraction, that is, quercetin-3-glucoside, *p*-coumaric acid, myricetin-3-glucoside, and myricetin-3-rutinoside. Moreover, total phenolic content in black currant seed residues was strongly correlated with antioxidant activity, suggesting that phenolic compounds contribute to their antioxidant capacities. Among the phenolic compounds identified in black currant seed residues, quercetin-3-glucoside, *p*-coumaric acid, myricetin-3-glucoside, and myricetin-3-rutinoside showed a strong positive correlation with antioxidant activities measured with both ABTS and DPPH radicals. Correlation analysis between oil and residue components (data not shown) led to the conclusion that no direct correlation exists between the two groups of components.

Principal component analysis (PCA) was performed to classify black currant cultivars on the basis of their seed oil composition and seed residue phenolic composition. Three principal components (PC) described 94% of the differences between cultivars. PC1 (40%) according to factor loadings (**Figure 2A**) was positively related to the content of cyanidin-3-rutinoside, seed oil, and linoleic acid and negatively related to the concentrations of total

sterols,  $\beta$ -sitosterol, *p*-coumaric acid, and linolenic acid. PC2 captured 31% of variables found between cultivars and described the concentrations of total phenolic, myricetin-3-glucoside, and antioxidant activity against the content of linolenic acid. PC3 (23%) related to the content of quercetin-3-glucoside and kaempferol-3-glucoside against total tocopherols,  $\alpha$ -tocopherol, and  $\delta$ -tocopherol concentrations. On the basis of the three PCs the five black currant cultivars were grouped into three clusters (**Figure 2B**). One cluster contained Ben Conan, Ben Alder, and Ben Nevis cultivars with high contents of total polyphenols, cyanidin-3-rutinoside, and linolenic acids, low content of *p*-coumaric acid, and low antioxidant activity. Ben Nevis formed an individual subgroup because of the highest concentrations of linolenic acid and oil in the seeds. The second group clustered Ben Sarek cultivar with the highest levels of total polyphenols, myricetin glycosides, total and  $\delta$ -tocopherols, and antioxidant activity and the lowest level of linolenic acid. Ben Tirran was a third individual cluster with the lowest oil, linolenic acid, and cyanidin-3-rutinoside contents and the highest total sterol and  $\beta$ -sitosterol concentrations. Multivariable data analysis revealed that the determination of health-related compounds

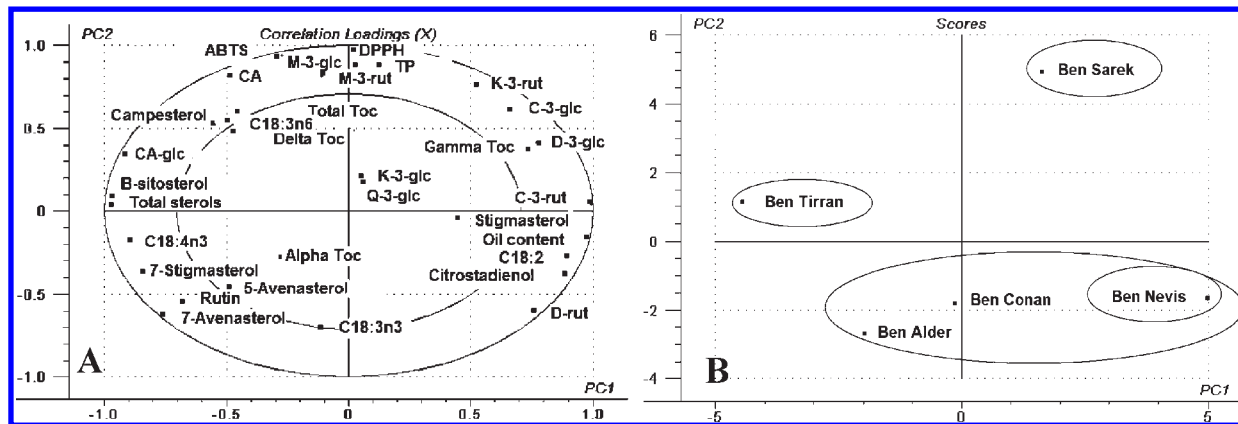


Figure 2. Principal component analysis of seed oil and residue of five black currant cultivars.

was suitable to discriminate among different cultivars of black currant berries.

The present study demonstrated that Canadian black currant seed oil is a good source of  $\gamma$ -linolenic and stearidonic acids, with considerable amounts of tocopherols and phytosterols. Black currant seed residues are byproducts of seed oil production and treated as low value waste. The high concentration of flavonols and *p*-coumaric acid in seed residues suggests that these may serve as dietary sources of natural antioxidants. Characterization of bioactive components in the black currant seed residues and demonstration of their potential beneficial properties may lead to value-added utilization of these berry seed residues and enhance the profitability of black currant production for the processing industries. The multivariable statistical analysis applied in this study was an efficient approach to evaluate and interpret easily the data and gave useful information on the investigated oil and residue characteristic of black currant seeds.

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Received for review June 23, 2009. Revised manuscript received October 27, 2009. Accepted November 10, 2009. Support from the Alberta Ingenuity Fund, Alberta Value Added Corporation Ltd., and Arden Delidais (D'nA Gardens) is greatly appreciated.