Effect of Genetic Modification on the Distribution of Minor Constituents in Canola Oil[1]

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ABSTRACT: Oil derived from different lines of genetically modified canola varieties was analyzed for phospholipids, tocopherols, and phytosterols by various chromatographic techniques. As observed previously in genetically modified soybean oils, there was a decrease in the content and composition of phosphatidic acid in three of the modified canola oils derived from the 12 varieties investigated. Normal-phase high-performance liquid chromatographic (HPLC) analyses showed small variations in the phospholipid content of major classes, despite few differences in their composition. Reversed-phase HPLC data indicated that the molecular species distribution of phosphatidylethanolamine was significantly altered by genetic modification when compared to phosphatidylcholine. Impact of oilseed modification on the tocopherol content was variable, with greater variation in the concentration of α- and γ-tocopherols than δ-tocopherol. Phytosterol composition was markedly affected by genetic modification. Brassicasterol, campesterol, and β-sitosterol levels were consistently lowered in one genotype, whereas increased brassicasterol content was observed in the other variety. In general, genetic modification of canola seeds led to changes in the distribution of phospholipids, tocopherols, and phytosterols.

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KEY WORDS: Canola oil, genetic modification, high-performance liquid chromatography, phospholipid class, phospholipid molecular species, phytosterol, tocopherol.

Minor bioactive constituents in vegetable oils include phospholipids (PL), tocopherols, and sterols. These substances are important by-products of the oil-processing industry. Mixtures of phospholipids, synonymous with commercial lecithin preparations, find many uses in the food industry as emulsifiers, stabilizers, and antioxidants (1). Tocopherols are the major ingredients in natural vitamin E products (2). Their antioxidative property and health benefits have been well recognized. Sterols are useful raw materials for the synthesis of hormones and related pharmaceuticals. Additionally, they provide valuable synthetic intermediates for many other medicinal applications (3).

Genetic modification of oilseeds produces a wide variety of oils with different fatty acid composition (4–8). Oxidative stability, functionality, and quality of vegetable oils can be affected by selective modification of the fatty acid composition (4,9–13), which consequently alters the distribution of minor bioactive components. In addition, market profitability of new modified oilseeds depends heavily on rich sources of minor constituents. In our continuous research directed toward assessment of the impact of oilseed modification on food oil quality and industrial utilization of vegetable oils (14), we studied the distribution of minor constituents of selected modified canola oils.

EXPERIMENTAL PROCEDURES

Materials. Experimental canola seeds of EX-varieties were obtained from InterMountain Canola company (IMC) (Cinnaminson, NJ). EX-100 and EX-200 varieties were commercially grown low-linolenic acid and high-oleic acid spring canola, respectively. The EX-600 variety was a commercially grown generic spring canola hybrid developed by Zeneca Seeds (Wilmington, DE). The EX-700 variety was commercially grown low-linolenic acid spring canola developed by University of Manitoba (Winnipeg, Manitoba, Canada). EX-900, EX-1000, and EX-1100 varieties were experimental IMC low-linolenic acid breeding germplasm. The EX-1000 canola oil was used as the control. Experimental transgenic canola seeds of DS-varieties were obtained from Calgene, Inc. (Davis, CA). The DS-68494 variety was used as the control.

Canola seeds (15 g) were ground (60 s) in a Varco coffee bean grinder Model 228 (Mouli Manufacturing Co., Belleville, NJ), and continuously extracted with hexane (200 mL) in a Soxhlet extractor for 5 h. The suspension was filtered through a homogeneous mixture of Celite filter aid (0.6) and charcoal (1.2 g) and washed with three 100-mL portions of hexane. The combined hexane extract was dried over anhydrous sodium sulfate (4 g) and filtered through folded filter paper. Removal of solvent under reduced pressure on a rotary evaporator at 25°C yielded crude oil which was stored in a freezer at –40°C.

Phospholipid standards were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). Phytosterol and tocopherol standards were obtained from Matreya, Inc. (Pleasant Gap, PA).

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Methods. Various chromatographic assays were performed with duplicate crude oil samples. A Thermo Separation Products Model SP4500 interface was used between a liquid chromatograph and a detector (see following relevant paragraphs for specifications and manufacturers). All except flame-ionization detector signals and output data were processed with Thermo Separation Products PC 1000 software for peak quantitation. Peak areas were mean values of triplicate injections, and analyte concentrations were determined by calibration with reference standards. Analytical data were validated by statistical analyses for computation of standard deviations and coefficients of variation.

Crude oil samples were analyzed for fatty acid composition by capillary gas chromatography (GC)–flame ionization detection. A Varian 3400 GC (Palo Alto, CA) was linked to a Supelco (Bellefonte, PA) capillary column (0.20 μm × 30 mm × 0.25 mm i.d.) packed with a Supelco SP2380 stationary phase. For sample analysis, the column temperature was initially maintained at 170°C for 10 min and then gradually increased to 220°C at 3°C/min. The injector and detector temperatures were set at 240 and 280°C, respectively.

Phospholipid analysis. Published procedures (14) were modified for the purification and analysis of phospholipids. Aliquots (5 g) of crude oil samples in duplicate were chromatographed on a silica gel column (10 g) and eluted with chloroform (200 mL) followed by 100 mL each of acetone, methanol, and methanolic phosphoric acid (0.1%). Combined methanolic eluates were evaporated under a nitrogen stream to leave a residue which was dissolved in chloroform. The chloroform solutions of enriched phospholipids were washed with 3 × 1 mL saturated sodium chloride solution, neutralized with sodium bicarbonate, dried over anhydrous sodium sulfate, and filtered. After removal of the solvent, the samples were ready for analysis by high-performance liquid chromatography (HPLC).

Purified phospholipid samples were analyzed for the composition and content of individual polar lipid classes by normal-phase HPLC. All analyses were carried out with a Thermo Separation Products (Fremont, CA) Model 8800 liquid chromatograph and a Varex Model ELSD II evaporative light-scattering (ELS) detector (Alltech Associates, Inc., Deerfield, IL). A MetaChem (Torrance, CA) Inertsil silica column (5 μm, 250 × 4.6 mm i.d.) was used throughout the HPLC analysis. Analytical samples (5–10 μL of 10 mg/mL solutions) were injected into the column *via* a Rheodyne (Cotati, CA) model 7125 injector (10–100 μL loop). The mobile phase employed a linear gradient elution starting from (A) chloroform/*t*-butyl methyl ether (75:15, vol/vol) to (B) methanol/ammonium hydroxide/chloroform (92:7:1, vol/vol/vol) in 30 min, staying at (B) for 10 min, and returning to (A) in 10 min at a flow rate of 0.5 mL/min.

Sufficient amounts of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were isolated for molecular species determinations by preparative normal-phase HPLC. In this mode, the same analytical mobile phase was pumped at a flow rate of 2 mL/min with a micrometric sample splitter set to deliver 1.2 mL/min for fraction collection. An Alltech LiChrosphere Si-60 column (10 μm, 250 × 10 mm i.d.) was used.

Molecular species of PC and PE were separated by reversed-phase HPLC (15). The Thermo Separation Products liquid chromatograph was interfaced with a Varex Model ELSD MK III detector for detection of analytes with enhanced sensitivity. Samples were injected into a Waters (Milford, MA) NovaPak column (4 μm, 300 × 3.9 mm i.d.) *via* a Rheodyne 7125 injector fitted with a 50-μL loop. Mobile phases for PC and PE were methanol/water/chloroform at ratios of 30:1:1 and 20:1:1, respectively. The flow rate was 0.8 mL/min.

Tocopherol analysis. Crude oil samples were analyzed for α-, β-, γ-, and δ-tocopherols by normal-phase HPLC-fluorescence detection. The Thermo Separation Products liquid chromatograph was interfaced with an Applied Biosystems, Inc. (Foster City, CA) Model 980 fluorescence detector set at 298 nm for excitation and 345 nm for emission. Solutions of crude oils in hexane (1:1, vol/vol) (16) were injected into a Waters μ-BondaPAK-NH_2 (300 × 3.9 mm i.d.) column *via* a Rheodyne 7125 injector fitted with a 20-μL loop. The column was eluted isocratically with a mobile phase of hexane/2-isopropanol (98:2, vol/vol) at a flow rate of 1 mL/min. In some cases, a mobile phase of hexane/*t*-butyl methyl ether (95:5, vol/vol) was used to separate closely packed components (17).

Phytosterol analysis. A Varian Model 3400 GC instrument equipped with a built-in flame-ionization detector was used in the simultaneous separation and quantitation of phytosterol components (18). Literature methods (19–21) were modified for sample preparation and derivatization. An aliquot sample of the crude oil (0.1 g) was stirred overnight with 1 N ethanolic potassium hydroxide (20 mL). The mixture was diluted with 20 mL water and extracted three times with ether (40 mL). The combined ethereal extract was treated with 0.5 N ethanolic potassium hydroxide and then washed with water until neutral. After removal of ether, the residue was dissolved in pyridine (0.25 mL) followed by the addition of bis(trimethylsilyl)-trifluoroacetamide (BSTFA) (0.25 mL). The mixture was heated for 15 min at 60°C and then left standing at room temperature overnight. Aliquots of the dried sample of trimethylsilyl phytosterols in methylene chloride were injected (275°C) into a Supelco capillary fused-silica column (30 m × 0.25 mm) coated with PTE5 (0.25 μm). The column temperature was initially held at 275°C for 20 min and then increased to 290°C at 3°C/min. The detector temperature was 300°C.

RESULTS AND DISCUSSION

Fatty acid compositional data for 12 canola oil samples are shown in Table 1. Among the oils in the EX-series, EX-200 and EX-1100 oils had the highest percentage of oleic acid

TABLE 1
Fatty Acid Composition (%) of Canola Oils

Sample	16:0	18:0	18:1	18:2	18:3	20:1
IMC varieties:						
*EX-1000[a]	3.8	2.0	66.4	18.0	6.9	1.4
EX-100	3.9	2.5	66.3	20.4	3.6	1.1
EX-200	3.2	2.5	81.3	6.5	4.1	1.5
EX-600	3.9	2.2	60.0	22.4	8.8	1.2
EX-700	3.7	2.3	66.3	23.1	2.1	1.1
EX-900	3.8	2.3	68.5	20.4	2.4	1.2
EX-1100	3.4	2.5	77.7	8.1	5.4	1.4
CG varieties:						
*DS68494	4.6	1.6	57.5	19.4	13.8	1.4
DS68456	4.3	1.4	56.5	15.5	10.6	1.0
DS68482	3.9	8.4	57.7	19.6	8.6	—
DS68507	3.7	22.0	38.8	19.7	12.7	—
DS68519	3.5	2.2	67.7	17.1	7.6	—

[a]EX-series: InterMountain Canola (IMC) canola oils. DS-series: Calgene (CG) canola oils. *EX-1000 and *DS68494 represent IMC and CG canola control samples, respectively. DS68456 contained 12:0 (8.7%). Coefficients of variation, 0.1–1.1%.

(18:1) (78–81%) but contained the least amounts of linoleic acid (18:2) (6.5–8.1%).

In the DS-series, the high-oleic oil DS68519 and high-stearic oil DS68482 had the lowest percentage of linolenic acid (18:3) (7.6–8.6%). In comparison with the control, the high-stearic oil DS68507 contained relatively low levels of 18:1 (38.8%).

Data presented in Table 2 indicate that the content of PE, PC, phosphatidylinositol (PI), and phosphatidic acid (PA) in the three oils EX-100, DS68456, and DS68519 was lower than that of the respective control oils EX-1000 and DS68494, although their compositional data showed few deviations from standard reference oils. However, both the content and composition of PA in EX-600 and those of PC in EX-1100 were lowered by genetic modification. Further, the analytical values of PA in the latter high-oleic oil were highest among the oils in the EX-varieties, regardless of a decrease in the PA content/composition of some modified oils in the series. In this regard, it was possible that some of the PA in the analyses might be attributable to phospholipase-catalyzed hydrolysis during grinding and extraction. As observed in soybean oils (16), genetic modification of certain canola oils from EX-lines tended to yield PA at reduced levels.

It is of interest to note that the major PE molecular species (Table 3) in the high-oleic EX-200- and EX-1100-oils were 16:0/18:2 (62–64%) and 18:2/18:2 (30–33%) species, whereas the high-oleic DS68519 oil had an additional major species 16:0/18:1 (33.5%). This species was also present in EX-100 and the high-stearic oil DS68507, but was absent in both control lines. It is of significance that this is the first report on the detection of 16:0/18:1 molecular species of PE in canola. The high-stearic oil DS68507 contained an appreciable amount of 18:0/18:1 (10.2%), which was not detected in most of the other oils assayed. The results in Table 3 also show that EX-600 and EX-700 oils contained 16:0/18:0-PE (20%) along with the most abundant 18:2/18:2 (55%) species. The distribution patterns of PE molecular species in these oils were different from the EX-control but were comparable to the DS-control.

The composition data in Table 4 demonstrate the effect of genetic modification on the distribution of PC molecular species. In general, the distribution of PC molecular species was affected by genetic engineering to a much lesser extent than that of PE species. However, a good correlation could be established between the fatty acid composition and PC molecular species. Thus, the high-oleic oils EX-200, EX-1100, and DS68519 had the 16:0/18:1 species as the major component (57–88%). The high-stearic oil DS68507 was the only oil that contained the 18:0/18:1 species (5.1%). This oil also had a relatively high percentage (21%) of the 18:2/18:2 species when compared to others in the series.

Analytical data for tocopherols are shown in Table 5.

TABLE 2
Analysis of Phospholipid Classes in Canola Oils[a]

	Composition (%)				Content (μg/g)				
Sample	PE	PI	PA	PC	PE	PI	PA	PC	Σ
IMC varieties:									
*EX-1000	35.6	12.6	10.8	41.1	18.5	6.5	5.6	21.3	51.9
EX-100	26.1	18.9	12.1	42.9	5.1	3.7	2.4	8.4	19.6
EX-200	24.3	15.6	11.6	48.5	11.2	7.2	5.3	22.3	46.0
EX-600	33.6	14.9	6.50	44.9	16.6	7.4	3.2	22.1	49.3
EX-700	30.1	15.1	8.40	46.4	15.1	7.6	4.2	23.4	50.3
EX-900	28.2	17.4	9.50	45.0	13.9	8.6	4.7	22.3	49.5
EX-1100	30.3	13.4	23.3	32.9	11.1	4.9	8.5	12.0	36.5
CG varieties:									
*DS68494	26.8	16.0	23.5	33.7	13.3	8.0	11.7	16.8	49.8
DS68456	26.5	19.1	18.7	35.7	5.2	3.8	3.7	7.0	19.7
DS68482	24.3	17.0	25.2	33.4	13.9	9.7	14.4	19.1	57.1
DS68507	27.4	15.5	23.7	33.3	11.7	6.6	10.1	14.3	42.7
DS68519	26.7	18.8	26.2	28.4	6.5	4.5	6.3	6.9	24.2
CV range (%)	1.5–6.3	1.2–7.0	1.6–7.5	0.8–6.5	0.4–6.9	0.3–3.2	0.6–7.8	0.2–4.9	0.5–8.0

[a]For abbreviations and sample codes, see Table 1. PE, phosphatidylethanolamine; PI, phosphatidylinositol; PA, phosphatidic acid; PC, phosphatidylcholine.

TABLE 3
Molecular Species of PE in Canola Oils

Sample	PE composition (%)				
	16:0/18:0	18:2/18:2	16:0/18:2	16:0/18:1	18:0/18:1
Standard soy	4.0	40.0	53.0	2.0	1.0
IMC varieties:					
*EX-1000	4.8	38.1	51.9	3.1	3.1
EX-100	1.4	19.9	54.0	24.7	ND[a]
EX-200	6.4	29.6	64.1	Trace	ND
EX-600	20.6	54.7	22.5	2.2	ND
EX-700	19.9	56.7	23.5	ND	ND
EX-900	8.2	62.5	28.9	ND	ND
EX-1100	5.4	32.8	61.8	ND	Trace
CG varieties:					
*DS68494	25.0	50.6	21.3	ND	ND
DS68456	19.1	54.6	26.2	Trace	Trace
DS68482	11.2	59.7	23.3	5.3	1.0
DS68507	4.8	29.7	43.5	11.9	10.2
DS68519	8.5	14.7	43.3	33.5	Trace
CV range (%)	2.5–8.3	0.5–7.3	0.3–6.7	2.0–8.2	5.5–9.0

[a]ND, not detected; CV, coefficient of variation. For other abbreviations and sample codes, see Tables 1 and 2.

Impact of oilseed modification on the content of tocopherols appeared variable without a distinctive trend. Greater variability was observed in the concentration of α- and γ-tocopherols than δ-tocopherol in the modified oils. Three of the DS-varieties and one of the EX-oils had higher levels of α-tocopherol than the respective controls, but the content of two of the EX-oils was somewhat lower than that of the EX-control. On the other hand, a slight decrease in the concentration of γ-tocopherol in a few modified samples in both series was observed. Apparently, genetic modification of oilseeds produced few changes in the δ-tocopherol content. Attempted analyses of the 12 varieties of canola oils for additional tocol components showed no evidence of the presence of tocotrienols in any of these oil samples.

TABLE 4
Molecular Species of PC in Canola Oils[a]

Sample	PC composition (%)				
	16:0/18:0	18:2/18:2	16:0/18:2	16:0/18:1	18:0/18:1
Standard soy	5.00	43.0	47.0	3.00	3.00
IMC varieties:					
*EX-1000	Trace[a]	5.20	36.9	57.8	ND
EX-100	ND	2.90	40.4	56.7	ND
EX-200	Trace	ND	11.3	87.8	ND
EX-600	Trace	7.70	39.9	52.1	ND
EX-700	Trace	3.30	39.6	57.1	ND
EX-900	ND	5.80	42.2	52.0	ND
EX-1100	ND	Trace	13.2	85.7	ND
CG Varieties:					
*DS68494	1.20	12.5	44.6	41.8	ND
DS68456	1.20	19.2	44.2	35.4	ND
DS68482	Trace	8.30	42.3	48.4	Trace
DS68507	Trace	20.5	45.4	29.1	5.1
DS68519	Trace	4.90	37.9	57.0	ND
CV range (%)	6.9–9.0	4.3–8.7	0.5–6.5	0.4–6.4	2.9–9.0

[a]For abbreviations and sample codes, see Tables 1–3.

TABLE 5
Analysis of Tocopherols in Canola Oils

Sample	Tocopherol content (μg/g)				
	α	β	γ	δ	Σ
IMC varieties:					
*EX-1000	214.5	ND[a]	445.4	5.4	665.3
EX-100	179.9	ND	295.2	3.3	478.4
EX-200	204.8	ND	405.0	6.8	616.6
EX-600	231.5	ND	278.4	4.1	514.0
EX-700	225.4	ND	446.6	4.7	676.7
EX-900	269.5	ND	373.3	5.9	648.7
EX-1100	188.0	ND	443.6	6.6	638.2
CG varieties:					
*DS68494	169.5	ND	376.8	5.5	551.8
DS68456	172.9	ND	472.6	5.8	651.3
DS68482	231.5	ND	373.6	6.5	611.6
DS68507	241.8	ND	381.3	9.5	632.6
DS68519	245.5	ND	335.2	6.8	587.5
CV range (%)	0.3–4.5		0.2–6.0	0.7–5.9	0.5–7.0

[a]For abbreviations and sample codes, see Tables 1 and 3.

As demonstrated in Table 6, phytosterol contents in crude canola oils were dramatically influenced by genetic modification of oilseeds. A significant decrease in amounts of the three major sterols (brassicasterol, campesterol, and β-sitosterol) in modified EX-oils was observed. The brassicasterol content ranged from 85 to 189 mg/100 g oil for modified oils, compared to 199.9 mg/100 g oil for the control. The campesterol content ranged from 205 to 264 mg/100 g oil for modified oils and was 421 mg/100 g oil for the control. The β-sitosterol content ranged from 457 to 509 mg/100 g oil for modified oils, as compared to 782 mg/100 g oil for the control. On the other hand, no systematic trend of impact was found in the phytosterol concentration of oils in DS-lines. The high-stearic oil DS68507 appeared to contain higher levels of phytosterols than the control. The concentration of campesterol and β-sitosterol in DS68456 was notably elevated, despite the fact that it had a similar fatty acid composition to the control

TABLE 6
Analysis of Phytosterols in Canola Oils

Sample	Phytosterol content (mg/100 g oil)			
	Brassicasterol	Campesterol	β-Sitosterol	Σ
IMC varieties:				
*EX-1000[a]	199.9	420.5	781.9	1402.3
EX-100	188.3	264.1	508.6	961.0
EX-200	188.9	222.9	473.7	885.5
EX-600	85.0	205.0	475.9	765.9
EX-700	149.7	248.9	474.5	873.1
EX-900	144.6	238.5	456.7	839.8
EX-1100	173.5	242.9	499.0	915.4
CG varieties:				
*DS68494	176.3	381.9	762.7	1320.9
DS68456	177.7	469.4	801.4	1448.5
DS68482	196.7	288.9	558.6	1044.2
DS68507	360.3	478.5	878.8	1717.6
DS68519	205.6	403.0	774.8	1383.4
CV range (%)	0.4–9.9	0.5–9.5	0.3–9.0	0.4–8.0

[a]For abbreviations and sample codes, see Tables 1 and 3.

DS68494. Conversely, the two phytosterols in DS68482 oil were present in lower concentrations compared to the control.

In conclusion, genetic modification of canola seeds produces oils in which the content and composition of minor constituents are different from conventional cultivars. Controlled modification of oilseeds can provide good sources of minor constituents with improved yields. No relationship was found between fatty acid composition and the distribution of phospholipid classes, tocopherol components, and phytosterols. In some instances, compositional data for PC molecular species correlate well with the fatty acid composition of the genetically modified oils. Correlation of PE molecular species with the fatty acid composition of the genetically modified oils suggests different biosynthetic pathways or intermediary biotransformations.

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