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Fatty Acids and Sterols in Oils from Canola Screenings[1]

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ABSTRACT

One sample of canola seed (variety Tower) and five samples of screenings were commercially processed to yield first an "expeller oil" and subsequently an "extractor oil" by the hexane extraction of the residue. The screening samples contained 25-50% intact or broken canola seed. The balance included 21-31% weed seeds (especially lambsquarter and stinkweed), hulls, fragments of the embryo, and chaff. All the oil samples were analyzed for sterol and fatty acid composition. The extractor screening samples had slightly higher sterol contents than the corresponding expeller samples, while the Tower samples gave the lowest values. The averages (in mg/g oil or extract) for the extractor screening samples were: brassicasterol, 1.0; campesterol, 4.1; and β-sitosterol, 7.3. For expeller screening samples the averages were: 0.9, 3.6 and 6.2, and for the Tower oils they were, respectively, 0.9, 3.8, 5.3 and 0.9, 3.5, 4.7. The fatty acid compositions of the screening samples for both extractor and expeller oils were similar to that of the Tower oil except for the higher proportions of docosenoic acid (22:1) and eicosenoic acid (20:1) and the more obvious presence of three C_{18} conjugated dienes totalling up to 0.6% of one screening oil sample. The docosenoic acid level (mainly erucic acid) ranged from 3.0 to 7.0% for the extractor oils and from 2.5 to 8.0% for the expeller samples, compared to 0.1% for the two Tower oils. The oil contents of the screenings ranged from 20 to 30%, and the fatty acids and sterols appear to be nutritionally useful and innocuous in all respects.

INTRODUCTION

Screenings are an inadvertent but economically significant factor in the canola (registered name for low-glucosinolate, low-erucic-acid varieties of *Brassica napus* or *Brassica campestris*) industry in western Canada. Farm deliveries of canola seed include damaged and immature canola seeds, and some genetically related seeds (e.g., mustard), but a variety of weed seeds are always included. The whole of this undesirable material may be termed dockage. A substantial portion can be removed, accompanied by some loss of sound canola seed, as screenings. This material is not normally processed in any way for oil or meal production. As part of a program to investigate the characteristics of screenings in animal nutrition, two types of oil were prepared from each of five sets of screenings, respectively denoted as extractor and expeller oils. These oils were examined for fatty acids and sterols. Following our earlier investigation of rapeseed oils for minor fatty acids (1), we have now applied the same examination technology, and our current results indicate generally unimportant differences between canola oil and screenings oils. The screenings oils, however, had appreciable erucic acid, whereas the Tower oils had only 0.1%. The screenings oils also had up to 0.6% total conjugated octadecadienoic acids, compared to only traces in the Tower oils.

EXPERIMENTAL PROCEDURES

The five lots of screenings, and one lot of canola seed, variety Tower (*Brassica napus*), from the 1977 crop were delivered to the P.O.S. Pilot Plant Corporation, Saskatoon. The lots amounted to ca. 900 kg each from five separate locations in three provinces. All were sequentially flaked, cooked, expelled and extracted (hexane) by conventional procedures (2). The oils were shipped to Halifax for analysis and were allowed to stand to settle out any fine solids present. Then the upper two-thirds to three-quarters of the oils was decanted into nigrogen-purged containers as the sample for analysis. A small lot of the original mixture of screenings (B) was obtained later. Seeds of sample B were crushed and extracted in the laboratory by boiling with hexane for 1 hr under nitrogen.

[1] Presented in part at the ISF/AOCS World Congress, New York, April-May 1980.

The oils were saponified, and nonsaponifiables were removed by AOCS procedure Ca-6a-40. The soaps were acidified and the fatty acids recovered and converted to methyl esters by refluxing for 1 hr in a solution of 7% BF_3 in MeOH under an atmosphere of nitrogen (3).

Analytical gas liquid chromatography (GLC) of the methyl esters was executed on wall-coated, open-tubular columns of stainless steel, 47 m in length and 0.25 mm id. The liquid phases were SILAR-5CP or -7CP. The apparatus was a Perkin Elmer Model 900 with flame ionization detector. An aliquot of each methyl ester solution was totally hydrogenated (4) and reanalyzed for total chain length determination as an aid to quantitation (5) and to reveal any abnormal components. Several samples of methyl esters were subjected to thin layer chromatography (TLC) on silicic acid containing silver nitrate, as described earlier (1). The recovered materials were reexamined by GLC and by ozonolysis (6,7).

Sterols were determined by the method of M. Kovacs (8), in which 0.1 g of oil was saponified in a centrifuge tube containing 1 ml 50% KOH and 4 ml 95% ethanol. The tube contents were boiled on a hot plate for 1 hr. The unsaponifiables were extracted 4 times into 5 ml of hexane after adding 2.5 ml of distilled water. The extract was concentrated and analyzed for free sterols with a Perkin-Elmer 2930B gas chromatograph equipped with flame ionization detector. The glass column (80 cm × 2 mm id) was packed with Gas-Chrom Q, 80/100 mesh, coated with 3% OV-17. The column was operated at 230 C with helium carrier gas at 40 ml/min. Injector and detector temperatures were 235 and 240 C, respectively. The internal standard for quantitation was 5α-cholestane, and all sterols were identified by comparing their retention times with authentic standards.

RESULTS AND DISCUSSION

Basically, the term "dockage" means all material removable by sieves and aspiration, plus inseparable conspicuous material, not in excess of grade tolerance, which is hand-picked from the screened sample. Dockage constitutes ca. 9% of the farm deliveries of canola. Screenings are, in effect, the practical consequence of reducing dockage in an effort to improve the grade of the canola seed.

The screenings evaluated in this study included 21.1-31.3% weed seeds. As shown in Table I, stinkweed and lambsquarter were generally the most important of these weed seeds, with green foxtail being equally important in sample E. Of the weeds, stinkweed and flixweed are of the Cruciferae family and are potential sources of docosenoic acid.

Although most weed seeds are either innocuous or are eaten by animals at very low proportions of their diets, several are known (9) to contain objectionable alkaloids (e.g., jimson weed, *Datura stramonium*). The subject of toxic weed seeds is kept under review (10), as are plant seeds generally (11,12); weed seeds are usually a relatively minor part of the problem of toxins in animal feeds (13). Of the weeds listed in Table I, only stinkweed is thought to be at times a nuisance when using screenings for animal feeding (14).

Not surprisingly, some 20-55% of the samples consisted of intact, immature, or broken seeds of canola or rapeseed. Along with these may be included small amounts of mustard seeds, of which the yellow (European = white) mustard *Brassica hirta* is distantly related to canola, whereas yellow seed (Oriental) and brown mustard are the *Brassica juncea* species and are more closely related to canola. Because of "volunteering" of seeds from earlier crops, a modest inclusion of rapeseed or mustard is not surprising in some fields. Wild mustard, *Sinapis kaber* (D.C.), is always a possibility. The balance of the samples consisted of chaff, unidentifiable fragments of plant material and dirt.

Table II compares the oil contents of the five samples of screenings with that of canola (var. Tower), and the acetone-insoluble materials in the two types of oils. The screenings contain one-half to two-thirds of the oil of the canola seed, but the proportion of actual canola seed included was not a

TABLE I

Percentage (by Wt) of Total Weed Seeds, and Breakdown by Major Species (26) in the Five Screening Samples[a]

	Samples				
	A	B	C	D	E
Total weed seeds	21.1	25.6	29.0	28.9	31.3
Composed of (%):					
Stinkweed, *Thlaspi arvense* L.	14.1	4.4	14.9	6.2	0.1
Lambsquarters, common, *Chenopodium album* L.	6.1	19.8	12.6	17.5	11.2
Flixweed, *Descurainia sophia* (L.) Webb	+	0.9	0.1	0.1	+
Redroot pigweed, *Amaranthus retroflexus* L.	-	+	-	-	0.4
Russian pigweed, *Axyris amaranthoides* L.	-	+	0.3	-	-
Green foxtail, *Setaria viridis* (l.) Beauv.	-	-	0.6	0.4	11.9
Chickweed, *Stellaria media* (l.) Cyprillo	0.1	+	+	-	-
Smartweed, *Polygonum sp.*	-	-	-	1.8	0.6
Flaxseed, *Linum usitatissimum* L.	-	-	-	-	4.4
Wild mustard (*Brassica kabar* (D.C.) L.C. Wheeler var. *pinnatifida* (stokes) L.C. Wheeler	-	0.3	-	0.8	1.4
Wormseed mustard, *Erysimum cheiranthoides* L.	-	+	0.2	0.2	0.2
Shepherd's purse, *Capsella bursa-pastoris* (L.) Medic	0.7	-	0.3	0.4	+
Canada thistle, *Cirsium arvense* (L.) Scop.	-	0.2	-	-	0.3
Catchfly, nightflowering + *Silene noctiflora* L.	+	+	+	1.0	0.3
Buckwheat, wild, *Polygonum convolvus* L.	-	-	-	-	0.5
Goosefoot, *Chenopodium sp.*	-	-	+	0.3	+
Sweetclover, yellow, *Melilotus officinalis* L. Lam.	-	-	-	0.2	-
Hawksbeard, narrowleaf, *Crepis tectorum* L.	0.1	-	+	-	-

[a]Data courtesy of Agriculture Canada.

TABLE II

Raw Material Composition, Oil Content and Acetone-Insoluble Material in Expeller and Extractor Oils, for One Lot of Canola Seed (var. Tower) and Five Lots of Screenings.[a]

	Tower	Samples				
		A	B	C	D	E
Composition (%)						
Rapeseed	99.3[b]	46.9	42.4	24.6	54.5	50.7
Weedseed	0.6[c]	21.1	25.7	29.0	28.9	31.3
Inert material	-	31.6	31.6	46.2	16.4	17.4
Oil content[d]	44.3	22.2	20.8	29.9	24.7	29.6
Acetone insoluble matter[e]						
Expeller oil	0.37	2.31	1.09	0.66	1.07	0.73
Extractor oil	1.31	2.44	1.69	1.16	2.20	1.96

[a]Data courtesy of J.A. Blake, POS Pilot Plant Corp., Saskatoon.

[b]2% *B. campestris* was found in this *B. napus* material.

[c]Wild mustard.

[d]As received, Foss-let method (AOAC 24 B03).

[e]National Standard of Canada CAN2-32.300M-76 test method 5.2.2, carried out on stirred material freshly obtained from equipment in POS operations.

factor in determining the oil content. The acetone-insoluble materials are not unusual for crude vegetable oils such as rapeseed or soybean oils (15,16), and the relationship of the higher values for the screenings to that of the Tower seed is basically a reciprocal relationship based on oil content. Crude degummed rapeseed oil (usually a mixture of 2-3 parts expeller oil and 1 part extractor oil) should have an acetone-insoluble (phosphatide) content $\leq 0.6\%$ (16); in the absence of degumming, these values are quite low. Expeller oil is usually ca. 0.8%, and extractor oil ca. 1.7% (J.A. Blake, private communication).

The important fatty acids of the screening samples (Table III) were similar to each other and differed from those of the Tower oil in that they had 3-8% of 22:1 and less 20:1—proportions much lower than those in conventional high-erucic-acid rapeseed oils (1,17). Lambsquarter could contribute 20:1 and 22:1 (18). Total important monoethylenic acids were somewhat less, and total polyethylenic acids somewhat more than in the Tower oil, but the figures for the latter were not very different from those of some recent *B. campestris* varieties, which had 35.0 and 36.8% total 18:2ω6 and 18:3 ω3 (17). Important saturated acids were slightly higher in the screenings oils than in the Tower oil. The monoethylenic isomer proportions are not given in Table III, but it is interesting to confirm our previous observation for rapeseed oils—that the proportion of 22:1Δ15 to 22:1Δ13 was inversely related to total 22:1 in the oil (1). Thus the Tower oil (0.1% 22:1) had 22:1Δ15 as 2.3% of total 22:1, whereas expeller oil A (8.0% 22:1) had 22:1Δ15 as only 0.5% of total 22:1. In samples A, C, D and E, 22:1 was slightly higher in the expeller oils than in the extractor oils. The significance of this probably lies in the ease with which triglycerides high in 22:1 were recovered in the expeller oils. Conversely sterols are higher in the extractor oils. Although none of the oils was degummed, the lengthy settling process would probably reduce the phosphatides in the portions of oil analyzed in Halifax and this separation could have a minor effect on some components. Phosphatides normally have very little 22:1 (17).

Conceivably, the higher (relative to that for Tower) 16:0 and 18:2ω6 in the screening oils could reflect inclusion of small or immature Tower seeds (19,20). However, the higher 22:1 masked possible complementary changes in 18:1 reported in the same reports.

In line with the major objective of the project, particular attention was paid to minor fatty acids. In most respects, these were similar in screenings samples and also were similar to the Tower oil, although 20:2ω6 and 22:2ω6 were definitely higher in the screenings oil. The average of 0.23% for the screenings samples compares with higher (0.32-0.64) values reported earlier for a high-erucic-acid rapeseed oil (21) and differs from an average of 0.07% in several canola oils (20); presumably, this reflects the elongation of C_{18} to C_{20} also observed in the monoethylenic acids.

Three minor components were observed with equivalent chain length (ECL) values of 19.68, 20.28 and 20.65 on SILAR-5CP. Upon argentation TLC, these components ran in the 22:1 region. Experience indicates that these two chromatographic properties are those of C_{18} conjugated diethylenic fatty acids (22). Comparison of SILAR-5CP and SILAR-7CP analyses (Fig. 1) provides further confirmation of conjugated octadecadienoic acids as detailed in recent investigations of several vegetable oils (22). The Tower seed oil contained only traces of the conjugated octadecadienoic acids, and the others contained variable, but more important, proportions (Table III). Screenings sample B had large amounts of one of these acids, as illustrated in Figure 1 for extractor oil. This acid was believed to be *trans*-9, *trans*-11 octadecadienoic acid. The other two were tentatively identified as *cis*-9, *trans*-11- and *trans*-10, *trans*-12-octadecadienoic acids, the latter being more important. The laboratory extract oil of sample B confirmed that the *trans*-9, *trans*-11 octadecadienoic acid was present at 0.5% of total fatty acids, and an isolate gave an ECL value of 20.59 on SILAR-7CP, further supporting a conjugated dienoic structure (22). These conjugated dienoic acids need to be investigated with more specific weed seed samples. In this particular set of samples, they are not artifacts of the oil preparation process—a possible source (22)—but must be natural components of the weed seeds or result from damage to canola seeds.

Artifact conjugated acids derived from linoleic acid usually retain one original *cis* bond, so that a *cis*-9 or *cis*-12 bond would be expected. The new bond in the Δ11 or Δ10 position can be either *cis* or *trans*. There is one well-established natural C_{18} di-*trans* conjugated fatty acid, *trans*-10, *trans*-12-octadecadienoic, found in *Chilopsis linearis* (family Bignoniaceae) seed oil as 5-10% of fatty acids, but this is a unique case (23).

The sterols previously obtained (24) from the same

TABLE III

Fatty Acids (w/w%) and Iodine Values of Expeller and Extractor Oils from One Lot of Canola Seed (var. Tower) and Five Lots of Screenings

	Tower		Samples									
			A		B		C		D		E	
	Expeller	Extractor	Expeller	Extractor	Expeller	Extractor	Expeller	Extractor	Expeller	Extractor	Expeller	Extractor
Important saturated acids												
16:0	4.09	4.23	4.12	4.48	5.63	5.73	4.36	4.73	5.60	5.82	4.65	4.85
18:0	1.47	1.53	1.41	1.75	2.07	2.12	1.51	1.59	1.70	1.73	1.72	1.74
20:0	0.50	0.55	0.46	0.49	0.69	0.89	0.56	0.54	0.56	0.56	0.51	0.60
22:0	0.29	0.32	0.21	0.27	0.36	0.40	0.28	0.27	0.37	0.37	0.32	0.33
24:0	0.09	0.10	0.06	0.09	0.11	0.20	0.10	0.11	0.14	0.18	0.16	0.13
Total	6.44	6.73	6.26	7.08	8.86	9.34	6.81	7.24	8.37	8.66	7.36	7.65
Important monoethylenic acids												
16:1ω9,7,5	0.25	0.23	0.34	0.32	0.52	0.55	0.30	0.36	0.36	0.42	0.26	0.34
18:1ω9,7	59.95	58.55	44.88	45.67	47.74	45.87	48.76	47.67	45.41	44.31	47.01	46.20
20:1ω9,7	1.32	1.35	3.76	3.78	1.86	2.63	2.91	3.06	2.23	2.31	2.78	2.79
22:1ω9,7	0.08	0.10	8.00	7.05	2.52	2.96	6.08	5.35	3.30	3.19	5.57	4.85
24:1ω9	0.14	0.17	0.90	0.81	0.33	0.46	0.57	0.51	0.49	0.49	0.26	0.20
Total	61.74	60.40	57.88	57.63	52.97	52.47	58.62	56.95	51.79	50.72	55.88	54.38
Important polyethylenic acids												
18:2ω6	20.42	21.42	23.45	23.33	25.66	25.84	22.23	23.47	27.71	28.24	23.16	23.89
18:3ω3	10.82	10.89	11.07	10.83	11.14	10.54	11.14	11.29	11.28	11.42	12.91	13.27
Total	31.24	32.31	34.52	34.16	36.80	36.38	33.37	34.76	38.99	39.66	36.07	37.16
Minor fatty acids												
16:3ω3	0.10	0.11	0.16	0.16	0.21	0.21	0.11	0.13	0.20	0.19	0.11	0.13
*t*9,*t*11-18:2	trace	trace	0.16	0.15	0.43	0.53	0.30	0.15	0.04	0.10	0.04	0.03
20:2ω6	0.05	0.07	0.44	0.36	0.11	0.18	0.29	0.27	0.18	0.19	0.12	0.16
Others[a]	0.37	0.36	0.54	0.55	0.58	0.73	0.46	0.51	0.42	0.46	0.41	0.42

[a]Includes, with ranges, 14:0 (0.005-0.11); 15:0 (0.02-0.04); 17:0 (0.04-0.10); 19:0 (0.03-0.06); 14:1 (0.01); 15:1 (0.01-0.06); 17:1 (0.05-0.08); 19:1 (0.02-0.10); 16:2ω6 (0.03-0.08), *c*9,*t*11-18:2 + *t*10,*t*12-18:2 (trace-0.05), 22:2 (trace-0.08); 20:3 (trace-0.06).

TABLE IV

Sterol Composition, Expressed As mg/g Oil, for Expeller and Extractor Oils from One Lot of Canola Seed (var. Tower) and Five Lots of Screenings[a]

	Tower		Samples									
			A		B		C		D		E	
Sterol	Expeller	Extractor	Expeller	Extractor	Expeller	Extractor	Expeller	Extractor	Expeller	Extractor	Expeller	Extractor
Brassicasterol	0.94	0.91	0.98	1.02	1.00	1.12	0.79	0.98	0.87	1.04	0.80	0.95
Campesterol	3.54	3.81	3.60	4.20	3.79	4.38	3.50	4.19	3.92	4.04	3.33	3.89
β-sitosterol	4.74	5.34	6.21	6.74	6.99	7.94	5.31	6.93	9.96	7.47	5.76	7.34

[a]Data courtesy of M.I.P. Kovacs, Agriculture Canada, Winnipeg.

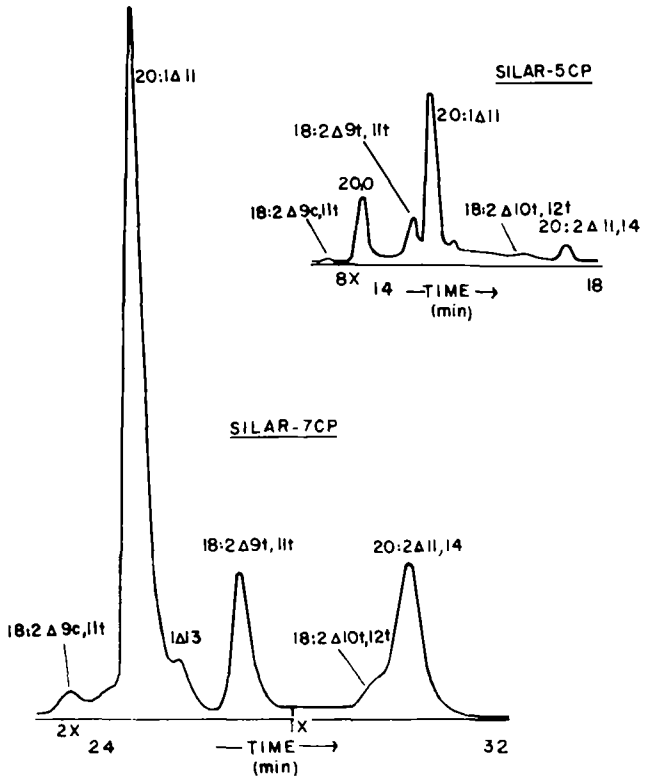

FIG. 1. Comparison of parts of gas liquid chromatographic analyses of extractor oil B. Note shift of significant component marked 18:2Δ9*t*,11*t* from before 20:1 on SILAR-5CP to after 20:1 on the more polar SILAR-7CP, with parallel shifting of other conjugated components.

variety of low-erucic rapeseed oil (Tower) can be averaged and were reported as mg/g brassicasterol, 0.7; campesterol, 2.8; and β-sitosterol, 4.4. The data from Table I averaged for the two Tower samples gave brassicasterol, 0.9; campesterol, 3.7; and β-sitosterol 5.1.

The extractor screening oil samples in all cases have higher sterol contents than the corresponding expeller samples. The averages for the extractor samples (in mg/g) were brassicasterol, 1.0; campesterol, 4.1; and β-sitosterol 7.3. These values were higher than those for the corresponding expeller samples (0.9, 3.6 and 6.2), and were also higher than the values for the two Tower oils (respectively, 0.9, 3.8 and 5.3, and 0.9, 3.5 and 4.7).

Free sterols are 0.3-0.4% of low-erucic-acid rapeseed oils, whereas sterol esters are 0.7-1.2% (25). The slightly higher recoveries of sterols in the extractor oils may reflect preferential extraction of the sterol in the form of esters occurring in some functional role such as membranes.

Monthly sampling of weed seed screenings from cereal crops showed that the seed composition could vary widely, but the oil composition was remarkably constant, although fatty acid details were not available (27). It may be concluded that the fatty acids and sterols of canola screenings are not sufficiently different from those of the canola seed to warrant concern about the nutritional effects if they were to be used as animal feed. The same conclusion would apply to the inclusion of admixed dockage in canola seed for crushing.

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