Effects of Processing and Storage on Chlorophyll Derivatives in Commercially Extracted Canola Oil

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This study characterizes the chlorophyll pigments present in canola oil immediately after commercial extraction and following oil storage to determine the best storage conditions for analytical samples and to examine the changes that chlorophyll derivatives undergo during oil processing and storage. Samples of pressed, solvent-extracted, crude and degummed canola oils, obtained from a commercial crushing plant, were stored for one month under four different conditions—in the freezer, in a refrigerator and at room temperature both in the light and in the dark. Chlorophyll derivatives (chlorophylls, pheophytins, pyropheophytins) were measured by high-performance liquid chromatography immediately after sampling and then on a weekly basis. The main pigments present in commercially extracted canola oil were pheophytin a, pyropheophytin a, chlorophyll a and chlorophyll b. The "a" derivatives comprised 81 to 100% of total chlorophyll pigments in the fresh oil samples. During degumming, the remaining chlorophylls were converted to pheophytins and pyropheophytins. During oil storage, exposure to light at room temperature affected the composition of chlorophyll derivatives as chlorophyll b was converted to pheophytin b and chlorophyll a was converted first to pheophytin a, then to pyropheophytin a.

KEY WORDS: Canola oil, chlorophyll, chlorophyll analysis, HPLC, pheophytin, pigments, processing, pyropheophytin, storage.

Every year, a portion of the Canadian canola crop is downgraded due to an unacceptable level of green immature seed. When the seed is crushed, the chlorophyll pigments are extracted with the oil, producing a dark-colored oil that is aesthetically unappealing to consumers. The chlorophyll pigments also act as photosensitizers, promoting oxidation of the oil and reducing its shelf life (1-5). Chlorophyll pigments act as catalyst poisons by blocking the active site of nickel catalysts and impairing hydrogenation (6). Chlorophyll can be removed from the oil by adsorption to bleaching clay, but as chlorophyll levels increase, more of the expensive bleaching clay is required. As larger amounts of clay are used, oil losses increase, because bleaching clay can retain 1/3 to 3/4 of its weight in oil (7).

Chlorophyll breakdown is poorly understood. The proposed initial steps in the chlorophyll breakdown pathway are illustrated in Figure 1 (8), and the structures of the chlorophyll derivatives are given in Table 1. The generic term "chlorophyll" is generally used to refer to all green pigments in canola seed, oil or meal. A previous study showed that canola seed contained mainly chlorophyll a (chl a) and chlorophyll b (chl b) in an approximate 3:1 ratio, while commercially extracted canola oil contained mainly pheophytin a (phy a) and pheophytin b (phy b) in an approximate 9:1 ratio (9). Seed quality also affected the chlorophyll/pheophytin composition, with moldy, heated or otherwise damaged seeds containing more pheophytins (10). Pheophytins have stronger prooxidant activity than chlorophylls (3) so pigment composition may have significant effects on the shelf life of the oil.

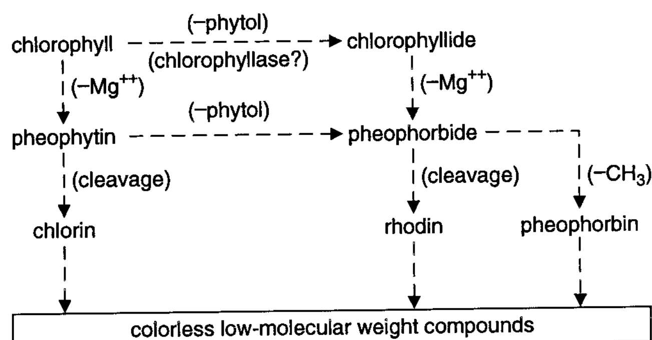

FIG. 1. Proposed initial steps in the chlorophyll breakdown pathway (Ref. 8).

Several studies have determined that pressed oils tend to contain fewer chlorophyll pigments than solvent-extracted oils (11-13). In recent studies (14,15), high-performance liquid chromatography (HPLC) was used to identify and quantitate the chlorophyll pigments present in canola seed, meal and commercially extracted oil. In at least one of the above-cited studies (14), the oil samples had been stored for a considerable period of time before analysis. The authors indicated that some chlorophyll pigments might have decomposed during storage. The first purpose of our study was to determine, both qualitatively and quantitatively, the chlorophyll pigments present in commercially extracted canola oil of various types immediately after sampling. The second purpose was to examine changes in these pigments during oil storage under various conditions.

MATERIALS AND METHODS

Freshly extracted canola oil samples were obtained from a western Canadian oilseed crushing plant. Samples were taken of pressed, solvent-extracted, crude and degummed oils. Sampling was repeated three times, resulting in three separate batches of oil that contained, by spectrophotometric analysis (16), 28, 54 and 79 mg kg^{-1} total chlorophyll, respectively. For the second two batches of oil, crude oil could not be obtained directly from the processor, and they were prepared in the laboratory from a 50:50 mixture of pressed and solvent-extracted oils. The fresh oil samples were placed in plastic bottles in a cooler and taken to the laboratory for immediate analysis. For the first batch of oil, the samples were refrigerated overnight prior to analysis. The second and third batches of oil were analyzed on the same day that they were collected from the crushing plant.

A subsample of each of the fresh oil samples was analyzed by HPLC to identify and quantitate the chlorophyll pigments present immediately after processing. The remaining oil was split into four treatments. For each

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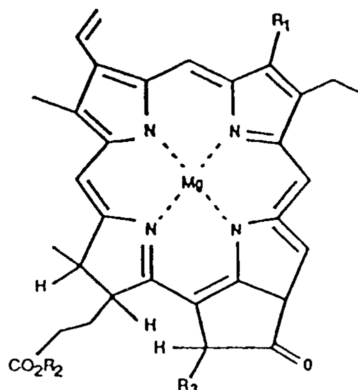
TABLE 1

Structure of Chlorophylls and Related Pigments



Pigment	Abbreviation	X	R_1	R_2	R_3
Pheophorbide a	Pho a	H_2	CH_3	H	CO_2CH_3
Methylpheophorbide a	Methyl a	H_2	CH_3	CH_3	CO_2CH_3
Chlorophyll b	Chl b	Mg	CHO	$C_{20}H_{39}$	CO_2CH_3
Chlorophyll a	Chl a	Mg	CH_3	$C_{20}H_{39}$	CO_2CH_3
Pheophytin b	Phy b	H_2	CHO	$C_{20}H_{39}$	CO_2CH_3
Pheophytin a	Phy a	H_2	CH_3	$C_{20}H_{39}$	CO_2CH_3
Pyropheophytin a	Pyro a	H_2	CH_3	$C_{20}H_{39}$	H

treatment, 20 mL each of the pressed, solvent-extracted, crude and degummed oils were placed in glass test tubes with screw caps. The oils were then stored for one month under four sets of conditions—in a freezer below −20°C in the dark, in a refrigerator at +10°C in the dark, on a bench at room temperature (~22°C) in the dark and on a bench at room temperature in the light. Dark storage was achieved by wrapping each test tube with aluminum foil to completely exclude light.

The oil from each test tube was sampled three times—after 8, 15 and 25 d of storage for the first batch of oil, and after 7, 14 and 28 d for the second and third batches of oil. Each sample was analyzed by HPLC to identify and quantitate the chlorophyll derivatives present to examine changes in these pigments during oil storage.

The oil was dissolved in acetone prior to analysis to give a solution of 25% oil. HPLC analysis was carried out according to the method of Endo *et al.* (14), except that the fluorescence detector was replaced with a photodiode array detector. The HPLC system consisted of two Waters model 510 pumps, a Waters model 715 Ultra Wisp sample processor and a Waters model 994 programmable photodiode array detector (Milford, MA). The column was stainless-steel (220 mm × 4.6 mm) packed with O.D.S. 5 μm (Pierce Chemical Co., Rockford, IL). Each batch of oil samples (fresh and stored) was run on the HPLC with a 50-μL injection volume and a run time of 30 min, which was sufficient to allow all of the chlorophyll derivatives to elute. The mobile phase was water/methanol/acetone (4:36:60) at a flow rate of 1 mL/min. The photodiode array detector was used to scan peaks to identify the chlorophyll pigments by their characteristic absorption maxima (Table 2). For the first batch of oil samples, quantitation was carried out at 410, 430, 450 and 490 nm. However, this resulted in a large carotenoid peak appearing in all chromatograms at a retention time of 2 to 5 min, which obscured pheophorbide a (pho a) and methylpheophorbide a (methyl a), both of which elute during this time period. For the second and third batches of oil, quantitation was carried out at 642, 655, 662 and 667 nm.

TABLE 2

Adsorption Characteristics of Chlorophylls and Related Pigments [E = Absorbtivity; (molar extinction coefficient)][a]

Pigment	Max. λ	E	Max. λ	E	Reference
Pheophorbide a	409	119200	667	55200	18
Methylpheophorbide a	408.5	122500	667	59200	17
Chlorophyll b	455	131000	645	47100	19
Chlorophyll a	430	94700	663	75000	19
Pheophytin b	434.5	145000	654	27800	18
Pheophytin a	409	101800	666	44500	20
Pyropheophytin a	409	102400	667	49000	19

[a]In acetone (except methylpheophorbide a in ether).

Chl a was purchased from Fluka Chemical Co. (Ronkonkoma, NY), chl b was purchased from Sigma Chemical Co. (St. Louis, MO) and the other pigments were prepared as described by Endo *et al.* (14). Calibration curves were prepared with standard solutions of chl a, chl b, phy a, phy b and pyropheophytin a (pyro a), as described in Endo *et al.* (14) who had previously shown that these five compounds were the major pigments that occur in commercially extracted canola oil. Standards were included with each set of samples, and chlorophyll pigments were identified by their absorption spectra and retention times compared to the standards. Standards were not prepared for pho a or methyl a, and both were quantitated from the phy a standard by multiplying with the ratio of the extinction coefficients (i.e., 1.24 for pho a and 1.33 for methyl a).

RESULTS AND DISCUSSION

The HPLC system separated pho a, methyl a, chl b, chl a, phy b, phy a and pyro a (Fig. 2). The small peaks that appeared immediately adjacent to the main peaks are due to epimers—for example chl a and chl a′, which were formed either during oil extraction, storage or analysis. Epimers were summed with the main pigments because there was no practical reason to consider them separately.

The major chlorophyll pigments detected in commercially extracted canola oil were phy a, pyro a, chl a, chl b and phy b. Small quantities of pho a, methyl a and epimers of chl b, chl a, phy b, phy a and pyro a also appeared in some samples. These results are basically in agreement with those of Endo *et al.* (14) and of Suzuki and Nishioka (15), who found the main pigments in crude and degummed canola oils to be phy a, pyro a, phy b and pyro b. The HPLC system in these experiments did not resolve pyro b from phy a, and any small amounts of this compound present would be included in the phy a results.

Effect of oil storage on chlorophyll derivatives. Duration of oil storage and storage conditions affected the composition of chlorophyll derivatives in the oil. In all types of oil, chl b was converted to phy b, and chl a was converted first to phy a, then to pyro a during storage. Conversion was most rapid in oils stored at room temperature in the light, followed by storage at room temperature in the dark and refrigerated storage, respectively. Only minor changes occurred in oil samples that were frozen (Table 3).

In the fresh oil samples, levels of phy a ranged from 21.2 to 62.9% of total chlorophyll pigments, pyro a from 4.1 to 55.3% and phy b from 0 to 12.9%. Significant levels of chl a and chl b were also detected in fresh oil samples, and these had not been reported by either Endo *et al.* (14) or Suzuki and Nishioka (15). Chl b levels ranged from 1.8 to 17.6% of total pigments, and chl a levels ranged from 5.0 to 37.2%, depending upon the type of oil tested. A possible reason for this discrepancy between our results and those of previous studies was that chlorophylls a and b were converted to pheophytins and pyropheophytins during oil storage. In the study by Endo *et al.* (14), the

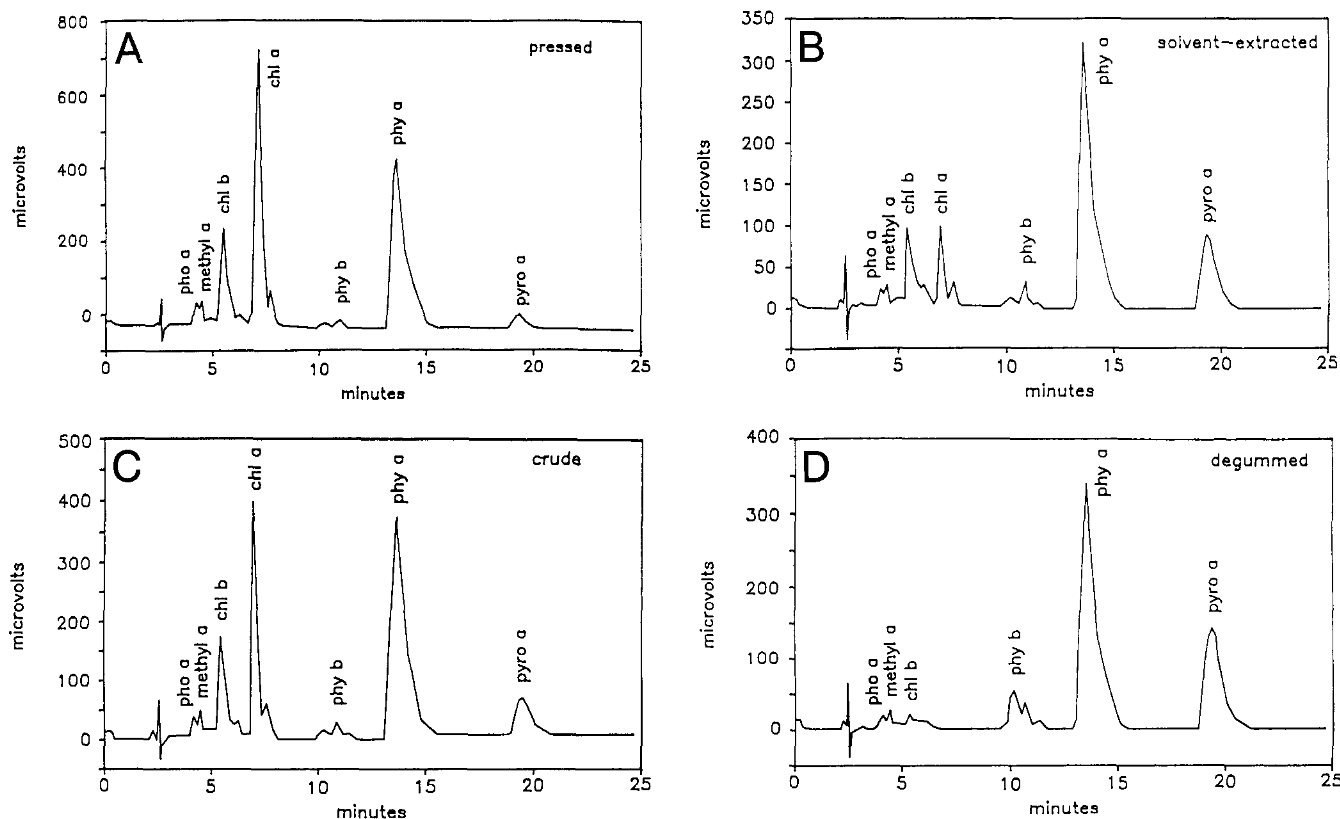


FIG. 2. Chlorophyll pigments present in (A) pressed, (B) solvent-extracted, (C) crude and (D) degummed canola oils stored in a freezer for 7 d. Abbreviations: pho a, pheophorbide a; methyl a, methylpheophorbide a; chl b, chlorophyll b; phy b, pheophytin b; chl a, chlorophyll a; phy a, pheophytin a; pyro a, pyropheophytin a.

TABLE 3

Changes in Chlorophyll Derivatives Present in Crude Canola Oil During Storage (for 28 d—batch 3)[a]

	Storage conditions				
Pigment	Fresh	Freezer	Fridge	Dark bench	Light bench
Chlorophyll b	14.9	13.2	11.2	7.4	1.7
Chlorophyll a	14.6	11.8	4.9	—	0.2
Pheophytin b	4.4	9.1	10.3	12.6	12.6
Pheophytin a	52.5	52.5	58.4	64.1	65.9
Pyropheophytin a	10.6	10.6	12.3	12.1	15.3

[a]79 mg kg^{-1} total chlorophyll pigments—expressed as percentage of total chlorophyll pigments.

TABLE 4

Percentage of "a" Derivatives in Fresh Oil Samples

Oil type	Batch 1 (28 mg kg^{-1})	Batch 2 (54 mg kg^{-1})	Batch 3 (79 mg kg^{-1})
Pressed	100.0	81.9	81.7
Solvent-extracted	89.6	86.9	82.4
Crude	87.6	84.5	80.7
Degummed	95.5	90.5	86.8

TABLE 5

Ratios of Chlorophyll Derivatives in Fresh Crude and Degummed Oils[a]

Oil type	Ratio	Batch 1	Batch 2	Batch 3
Crude	chl b/phy b	All chl b	14.5:1	3.4:1
Degummed		All chl b	0.61:1	0.14:1
Crude	chl a/phy a	1.7:1	0.77:1	0.28:1
Degummed		All phy a	All phy a	All phy a
Crude	phy a/pyro a	0.75:1	1:1	5:1
Degummed		1.9:1	1.9:1	2:1

[a]See Table 1 for abbreviations.

samples had been stored for up to two years under cool, dark conditions prior to analysis, and the study by Suzuki and Nishioka (15) did not specify how long the commercially extracted oil was stored. The minor components detected included pho a, present at 0.2 to 1.4% of total chlorophyll pigments in fresh oil samples, and methyl a, present at 0.2 to 3.7% in fresh oil samples, as well as epimers of all five of the main pigments. These results are in agreement with those of Endo *et al.* (14) who reported traces of pho a and methyl a, and both Endo *et al.* (14) and Suzuki and Nishioka (15) reported traces of various epimers.

Effects of oil processing on chlorophyll derivatives. Suzuki and Nishioka (15) reported a lower proportion of a:b derivatives in solvent-extracted oil than in pressed oil, while Endo *et al.* (14) reported similar compositions. In our study, the proportion of a:b derivatives was quite variable. In batch one, the solvent-extracted oil contained a lower proportion of "a" derivatives than the pressed oil; in batch two, the solvent-extracted oil contained more "a" derivatives than the pressed oil; and in batch three, the two were almost identical (Table 4).

The predominant chlorophyll pigments detected in fresh-pressed, solvent-extracted and crude oils were phy a, pyro a, chl a, chl b and, in some cases, phy b. In the fresh degummed oils, chl a and chl b were either absent or present as minor components, while the main pigments detected were phy a, pyro a and phy b (Fig. 2). Therefore, degumming caused chlorophylls to be converted to pheophytins. Endo *et al.* (14) concluded that commercial oil extraction converted chlorophylls to pheophytins and that degumming converted pheophytins to pyropheophytins. They found a higher ratio of pyro a/phy a in the degummed oil than in the crude oil. We did not observe this. In our results, degumming converted chlorophylls to pheophytins, but there was no apparent conversion of pheophytins to pyropheophytins. We observed decreases in the ratios of chl b/phy b and chl a/phy a, and an increase in the ratio of phy a/pyro a between the crude and degummed oils (Table 5), with the exception of batch three, where the phy a/pyro a ratio was lower in the degummed oil than in the crude oil. Differences in processing conditions, such as cooking temperature and duration, might account for the differences between our results and those of Endo *et al.* (14). Suzuki and Nishioka (15) found that high cooking temperatures converted pheophytins to pyropheophytins. The duration and conditions of oil storage prior to analysis in Endo's study might explain the observed conversion of pheophytin to pyropheophytin.

The "a" type pigments comprised 81 to 100% of total chlorophyll pigments found in fresh commercially extracted canola oil (Table 3). This is in agreement with the results of Suzuki and Nishioka (15) who reported a b/a ratio of 0.2, and with the results of Endo *et al.* (14), who found 90% of the pigments in crude and degummed canola oils to be of the "a" type.

The type of oil and the total chlorophyll content of the oil both affected the proportion of a/b derivatives detected. For the three batches of oil that we examined, which averaged 28, 54 and 79 mg kg^{-1} total chlorophyll, the lower the total chlorophyll content of the oil, the larger proportion of "a" derivatives it contained. Fresh oil samples from batch one (28 mg kg^{-1}) contained 88 to 100% "a" derivatives, batch two (54 mg kg^{-1}) contained 82 to 91% "a" derivatives, and batch three (79 mg kg^{-1}) contained 81 to 87% "a" derivatives. In batches two and three, the degummed oil contained a higher proportion of "a" derivatives than the pressed, solvent-extracted or crude oils (Table 4). This can be explained by the conversion of chlorophylls to pheophytins and pyropheophytins during degumming, which requires acidic treatments. Chl b would be converted to phy b and pyro b, which we were unable to resolve. This likely accounts for the apparently lower proportion of "b" derivatives detected in degummed oils.

Pressed oils generally contained a higher proportion of chl a and chl b and less phy b and pyro a than did solvent-extracted oils. Therefore, solvent extraction may convert chl b to phy b and chl a to phy a and pyro a. However, unlike Suzuki and Nishioka (15), who found solvent-extracted oil to contain mainly pyropheophytins, we observed all five of the main pigments (chl b, chl a, phy b, phy a and pyro a) in solvent-extracted oils. Therefore, solvent extraction is likely responsible for some, but not all, of the chlorophyll pigment conversion that occurs during oil processing.

The amounts and composition of chlorophyll derivatives present in commercially extracted canola oil have important implications for bleaching. There is no information on the relative ease of removal of chlorophylls vs. pheophytins, but Suzuki and Nishioka (15) have found that, with activated earth, phy a and pyro a were removed six times more readily than phy b or pyro b. Results differed with activated carbon, however. Therefore, knowledge of the composition of chlorophyll derivatives in canola oil will make it easier to choose the correct amount and type of bleaching earth for efficient color removal. Knowledge of how extraction and processing conditions cause these pigments to interconvert should eventually allow us to manipulate processing conditions to yield an oil that can be bleached efficiently.

ACKNOWLEDGMENTS

Financial support was provided by the Canadian Wheat Board and the University of Manitoba. Staff at the Grain Research Lab of the Canadian Grain Commission provided materials, equipment and assistance; contribution No. 960 and 710. Oil samples were generously supplied by CanAmera Foods.

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[Received December 9, 1993; accepted April 28, 1994]