# Behavior of Chlorophyll Derivatives in Canola Oil Processing

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**Chlorophyll derivatives in canola oil were analyzed quantitatively by reversed-phase high-performance liquid chromatography without any pretreatment. The main components were pheophytin (pheo) a and b and pyropheophytin (pyro) a and b. The factors affecting the types and concentration of chlorophyll derivatives in oil have been investigated during seed preparation, expelling, extraction, degumming and alkali-refining processes. Bleaching tests of alkali-refined canola oil with activated earth indicated the adsorption of each derivative to decrease in the following order: pheo a > pyro a >> pheo b > pyro b. In bleaching with activated carbon, however, the following order was observed: pyro b > pheo b > pheo a > pyro a.**

KEY WORDS: Adsorption isotherms, bleaching, canola oil, chlorophyll derivatives, pheophytin a, pheophytin b, pyropheophytin a, pyropheophytin b, refining process, seed preparation.

For most vegetable oils, chlorophyll derivatives are undesirable substances for oil quality (1–5). These derivatives must be removed by adsorbents, such as activated earth (AE) or activated carbon (AC), in the bleaching process. There have been recent reports (6–15) on the occurrence of chlorophyll derivatives in canola seed and canola oil. Analytical methods for these derivatives have rapidly improved (12,15). There has been little research, however, on the behavior of these derivatives during canola seed and canola oil processing. The purpose of our investigation was (i) to select analytical conditions for the high-performance liquid chromatography (HPLC) separation and detection of chlorophyll derivatives in canola oil; (ii) to follow the changes of chlorophyll derivatives during canola seed crushing and canola oil refining; and (iii) to study behavior of chlorophyll derivatives in bleaching and their bleachability by adsorbents.

## MATERIALS AND METHODS

*Materials.* Chlorophylls a and b were purchased from Sigma Chemical Co. (St. Louis, MO). Pheophytins (pheo) a and b were prepared from chlorophylls a and b by reaction with acid (16). Pyropheophytins (pyro) a and b were prepared according to the method described by Fraser and Frankel (11).

*HPLC.* Chromatography was carried out on a system consisting of a Nippon-Bunkou (Tokyo, Japan) programmable PU-980 pump, a Nippon-Bunkou VL-614 injection system and a Nippon-Bunkou 821-FP fluorescence detector. Chlorophyll derivatives were determined at emission wavelength of 655 nm and excitation wavelength of 430 nm. The HPLC column was a stainless-steel cartridge, 5 mm × 20 mm, packed with ODS. The mobile phases and gradient program followed the method reported by Daun and Thorsteinson (12) or Endo *et al.* (15).

*Spectrophotometry.* Spectrophotometric measurements were made by official AOCS Method Cc 13d-55 (17).

*Extraction of oil.* Seeds (cotyledon without hull; 10 g) or hull (10 g) were powdered in a coffee mill, then extracted with hexane in a Soxhlet extractor for 8 h.

## RESULTS AND DISCUSSION

*Analytical HPLC conditions for determination of oil with low chlorophyll derivative content.* HPLC analyses of chlorophyll derivatives contained in canola oil were studied by Daun and Thorestein (12) and Endo *et al.* (15). In these studies, two types of mobile phase and two types of detector were used for HPLC analysis (Table 1). We have done comparisons of these analytical conditions to ensure reliable results in the direct analysis of alkali-refined and bleached canola oils that contained small amounts of chlorophyll derivatives. As a result of these comparisons, we drew the following conclusions: (i) The three-solvent system showed good separation of each peak (Table 1); (ii) the fluorescence detector showed good sensitivity in direct determination of low concentrations of chlorophyll derivatives (Table 1). As a result of this study, the analytical conditions shown in Figure 1 were selected for HPLC analysis of crude, degummed, alkali-refined and bleached oils.

*Determination of chlorophyll derivatives in canola oil.* HPLC of commercial alkali-refined oil from No. 1-grade Canadian canola seed showed four large peaks and five small peaks (Fig. 2). The large peaks (Nos. 4, 6, 7 and 9) were identified as pheo b, pyro b, pheo a and pyro a, respectively, by comparison with the retention times of authentic derivatives. Among the five minor peaks (Nos. 1, 2, 3, 5 and 8), Nos. 5 and 8 were regarded as pheo b′ and pheo a′, respectively, because of their nearness to the retention times of pheo b and pheo a (15). But these peaks need further investigation for definite identification. Nos. 1 and 2 were unknown. No. 3 corresponds to chlorophyll a, but its concentration was small. The concentrations of pheo a and pyro a were 6.5 ppm, pheo b was 1.3 ppm and pyro b was 1.1 ppm. The total content of these four components was 15.4 ppm. This was 1.4 times higher than determined by the AOCS method (17). This difference is due to the fact that the AOCS method was developed with chlorophyll a as a calibration standard. Only traces of chlorophyll a are present among the chlorophyll pigments in alkali-refined canola oil (12, J.K. Daun, personal communication). The b derivatives/a derivatives ratio was about 0.2, and the pyro derivatives/pheo derivatives ratio was about 1.0.

*Chlorophyll derivatives in cotyledon and hull.* The extracted oils from dehulled yellow cotyledon, dehulled green cotyledon and hull showed very different contents of each chlorophyll derivative (Fig. 3). Dehulled green cotyledon showed an extremely high level of chlorophyll derivatives, of which pheo a was more than half, and a remarkable amount of chlorophyll a was present as well. Dehulled yellow cotyledon had the lowest amount of chlorophyll derivatives, and hull was intermediate. There was no large difference in the b/a ratio between yellow and green cotyledon. On the other hand, the b/a ratio of hull was higher than that of cotyledon.

*Influence of heat in the conditioning process.* Usually canola seed is heated in a conditioning process prior to

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TABLE 1

**Comparison of Two Analytical Methods in High-Performance Liquid Chromatography of Chlorophyll Derivatives[a]**

| Solvent | Detector | Wavelength[c] (nm) | Chromatogram[b] Sensitivity | Chromatogram[b] Peak separation | Application |
|---|---|---|---|---|---|
| A: Acetonitrile[d] | Visible | 670 | △ | ● | |
| B: Isooctane/dichloromethane (1:1)[b] | | 655 | △ | ● | |
| | | 410 | ○ | ● | |
| C: water/methanol (1:9)[d] Gradient 1 mL/min[d] | Fluorescence | Em 670 Ex 405 | ● | ● | ● |
| | | Em 655 Ex 440 | ● | ● | ● |
| Acetone/10% water-methanol (60:40) 1 mL/min[e] | Visible | 670 | △ | ○ | |
| | | 655 | △ | ○ | |
| | | 410 | ○ | ○ | |
| | Fluorescence | Em 670 Ex 405 | ● | ○ | |
| | | Em 655 Ex 440 | ● | ○ | |

[a]Column: ODS 5 μM, 4.6 mm × 250 mm.
[b]●, Best; ○, better; △, good.
[c]Em, emission; Ex, excitation.
[d]Reference 12.
[e]Reference 15.

extraction of oil. Johansson and Appelquist (8) and Daun (9) implied changes in the chlorophyll components on heating from changes in the spectra of the extracted pigments. In our study, we measured which types of chlorophyll derivatives are dominant components at high temperature, such as in the cooking process. The chlorophyll derivatives' content gradually increased with increasing temperature and markedly increased when seed was heated at 110°C for 60 min (Fig. 4). The b/a ratio gradually increased with increasing temperature and prolongation of heating time. The pyro/pheo ratio markedly increased by heating above 110°C. This means that pheo a and b were changed to pyro a and b in seed tissues by the influence of heat, and that a low level of chlorophyll derivative in crude oil is achieved when exposure to heat is minimized during the conditioning process.

*Changes in crushing and refining.* The changes of chlorophyll derivatives in expelling, extraction, degumming and alkali-refining processes are shown in Figure 5. These oils were produced in a commercial plant from No. 1-grade Canadian canola seed. Extracted oil showed a higher level of chlorophyll derivatives than of pressed oil.

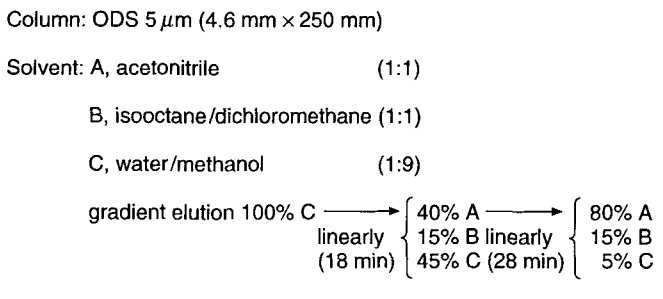



**FIG. 1. High-performance liquid chromatographic analytical conditions. Em, emission; Ex, excitation.**

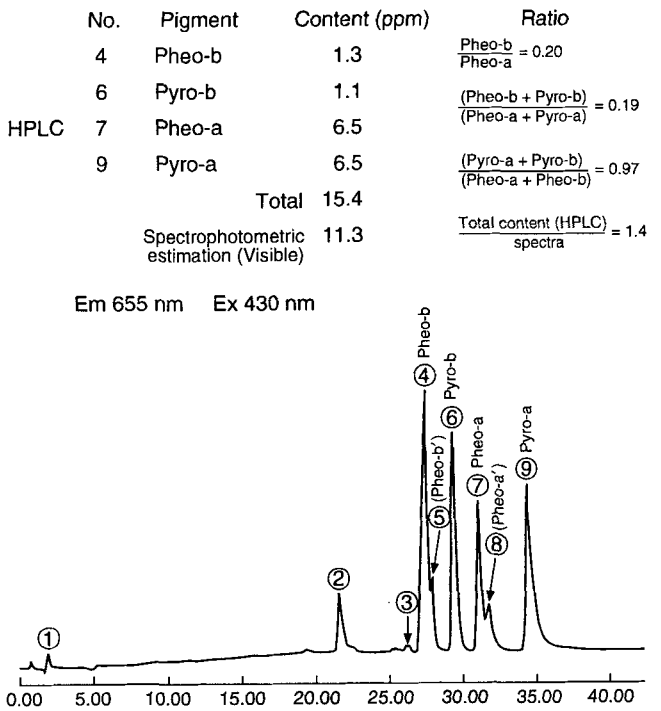



**FIG. 2. Example of high-performance liquid chromatographic (HPLC) chromatogram of alkali-refined canola oil. Em, emission; Ex, excitation; Pheo, pheophytin; Pyro, pyropheophytin.**

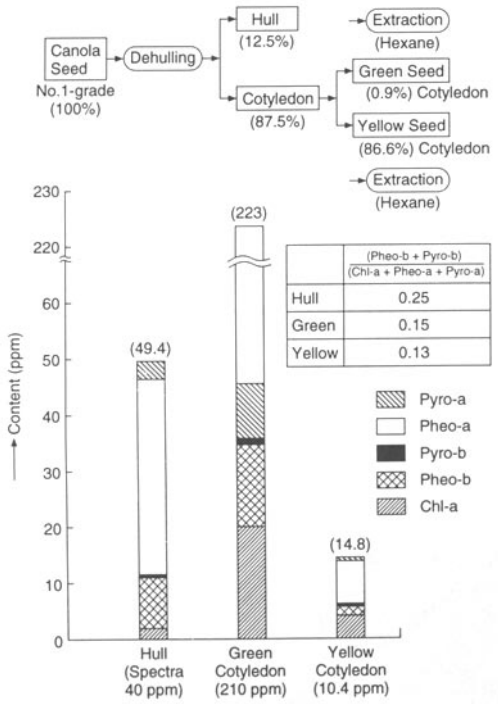



**FIG. 3. Chlorophyll derivatives in cotyledon and hull of canola seed. Abbreviations as in Figure 2. Chl, chlorophyll.**

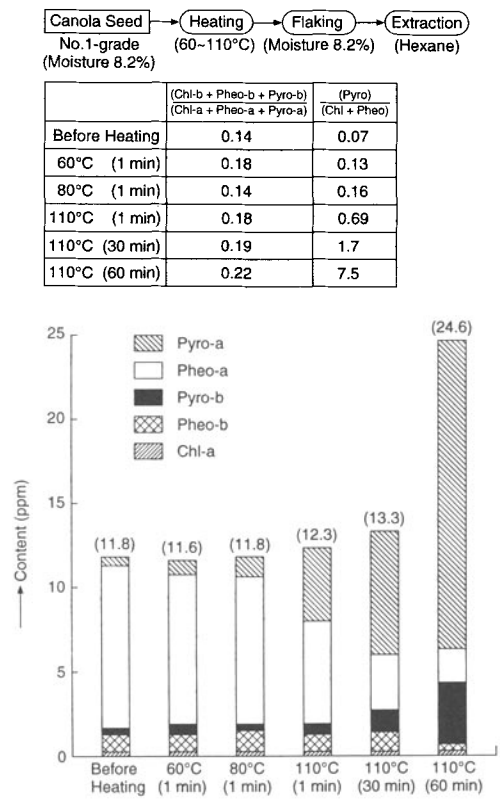



**FIG. 4. Effect of heat on chlorophyll derivatives in seed. Abbreviations as in Figure 3.**

| | (Pheo-b + Pyro-b) / (Chl-a + Pheo-a + Pyro-a) | (Pyro-a + Pyro-b) / (Chl-a + Pheo-a + Pheo-b) |
|---|---|---|
| Pressed oil | 0.12 | 0.40 |
| Extracted oil | 0.20 | 3.6 |
| Pressed + extracted | 0.17 | 1.4 |
| Degummed oil | 0.16 | 1.6 |
| Alkali-refined oil | 0.18 | 1.4 |

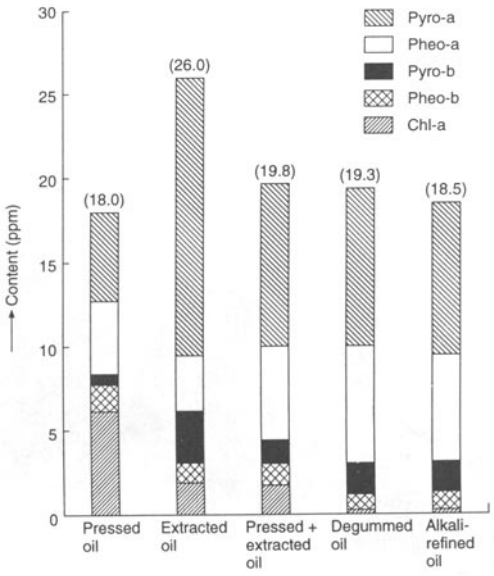



**FIG. 5. Changes of chlorophyll derivatives in canola oil from seed pressing to alkali-refining. Abbreviations as in Figure 3.**

Main components in extracted oil were pyro a and b. The b/a ratio in extracted oil was higher than that of pressed oil. Total content of chlorophyll derivatives decreased slightly during degumming and alkali-refining. Chlorophyll a was decreased during degumming (acid degumming). There were no large changes in the b/a ratio or the pyro/pheo ratio during degumming and alkali-refining.

*Bleaching tests.* The behavior of chlorophyll derivatives in the bleaching process is shown in Figures 6 and 7. In Figure 6, chlorophyll derivatives remaining in bleached oil were measured by the official AOCS method (17); hence, each derivative was not measured separately. In Figure 7, each derivative was measured separately by the HPLC method outlined earlier. The bleaching tests are summarized in Figure 8, for which the Freudlich isothermal adsorption equation was used. In this equation, x is the amount of chlorophyll derivatives adsorbed, m is the amount of adsorbent, C represents the residual derivative concentration and K and n are constants. The data in Figure 8 show the following: (i) K-values of pheo a and pyro a in AE bleaching are about six times larger than those of pheo b and pyro b, respectively. This means that pheo a and pyro a are more adsorptive than the corresponding b derivatives. Pheo b or pyro b may require about a six times larger amount of AE than that required for pheo a or pyro a in bleaching; (ii) On the other hand, in AC bleaching the K-value of pheo a is slightly smaller than that of pheo b, and the K-value of pyro a is half that of pyro b. This means that a derivatives are more difficult to remove than b derivatives in AC bleaching; (iii) There are no great differences in K-values between pheo a and

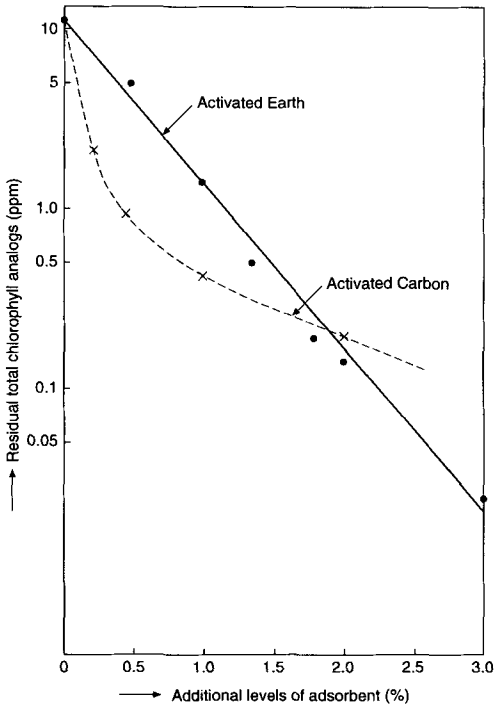
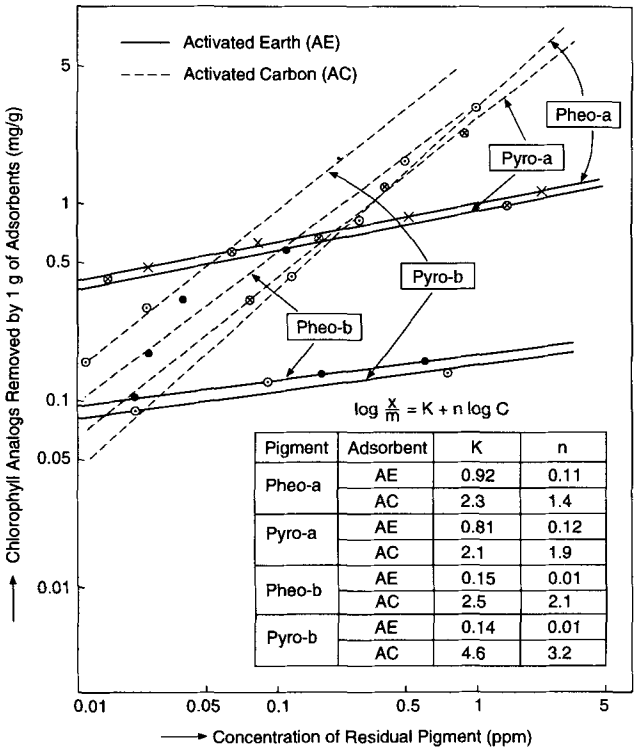



**FIG. 6. Bleaching tests of canola oil. I. Spectrophotometric analysis. Bleaching conditions: 80°C, 30 min, 30 mm Hg.**




| Pigment | Adsorbent | K | n |
|---|---|---|---|
| Pheo-a | AE | 0.92 | 0.11 |
| | AC | 2.3 | 1.4 |
| Pyro-a | AE | 0.81 | 0.12 |
| | AC | 2.1 | 1.9 |
| Pheo-b | AE | 0.15 | 0.01 |
| | AC | 2.5 | 2.1 |
| Pyro-b | AE | 0.14 | 0.01 |
| | AC | 4.6 | 3.2 |

**FIG 8. Adsorption isotherms of four chlorophyll derivatives. Abbreviations as in Figure 3.**

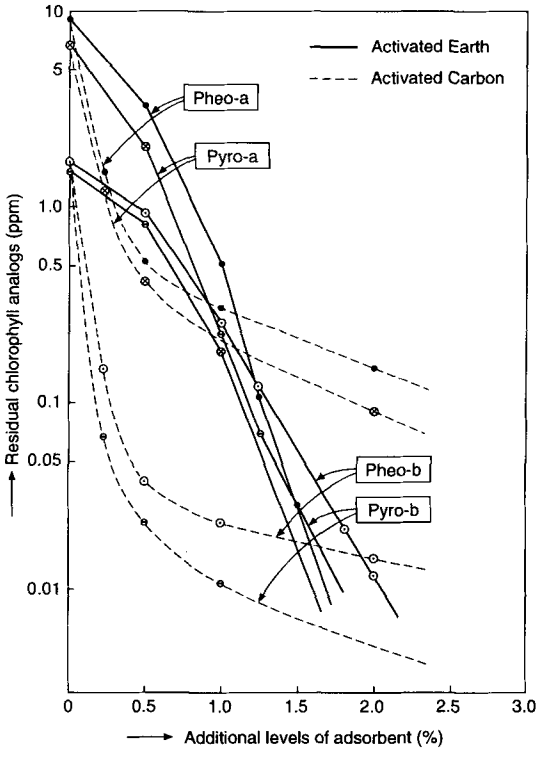



**FIG. 7. Bleaching tests of canola oil. II. HPLC analysis. Bleaching conditions as in Figure 6; abbreviations as in Figure 3.**

pyro a or pheo b and pyro b in AE bleaching. But the K-value of pyro b in AC bleaching is about two times larger than that of pheo a; (iv) AE is a suitable adsorbent for removal of a derivatives, whereas AC is more suitable for b derivatives; and (v) These tendencies of derivatives to adsorb on AE or AC are not consistent with the extent of the polarities of these derivatives. Because b derivatives are more difficult to adsorb on AE, we suppose that the formyl group at the R-3 position has something to do with the adsorbent/adsorbate interaction. But a more detailed examination is required.

*Spectral changes in bleaching.* Figure 9 shows the photometric spectral changes of canola oil observed in bleaching tests with AE or AC. The position of the absorption maximum of alkali-refined oil was at 671 nm. This is consistent with the absorption maximum of pheo a and pyro a. This maximum was shifted from 671 to 650≈660/nm during AE bleaching (18), but it was not shifted by AC. The reason for this shift may be attributed to differences in the adsorbabilities between a and b derivatives on AE. In AE-bleached oil, pheo b and pyro b (657 nm) were the dominant components, whereas the dominant components in AE-bleached oil were pheo a and pyro a, which have their absorption maximum at 671 nm, the same as alkali-refined oil. Therefore, no changes occurred upon AC bleaching in the wavelength of maximum absorption as compared to alkali-refined oil.

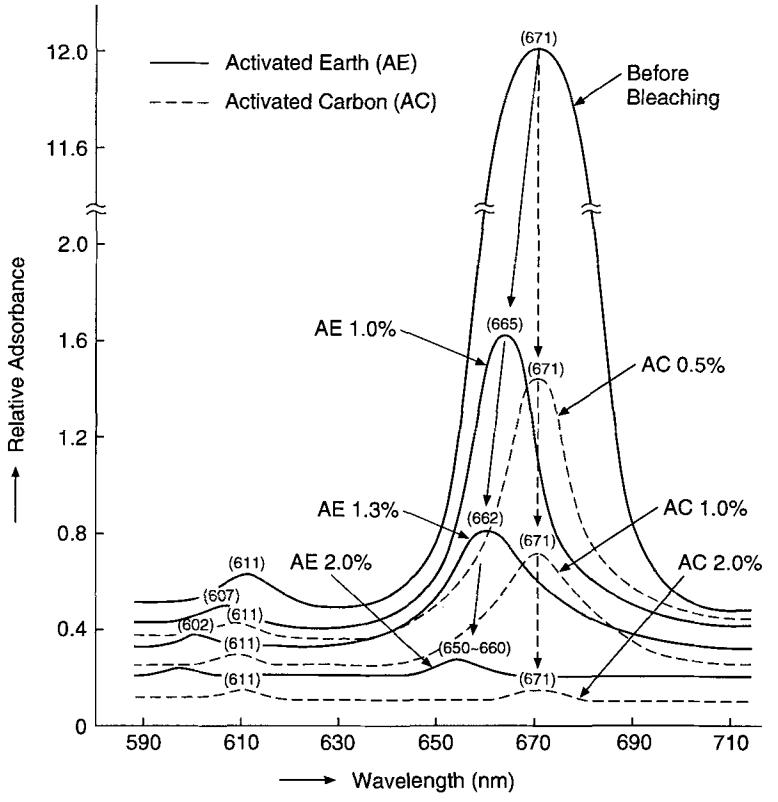



**FIG. 9. Spectral changes of canola oil in activated earth or activated carbon bleaching.**

## ACKNOWLEDGMENT

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