Separation and Characterization of Pigments from Bleached and Deodorized Canola Oil

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An analysis of pigments responsible for color formation during bleaching and deodorization of canola oils treated with activated bleaching earth (ABE) or novel mineral-acid/silica (AS) adsorbents is presented. The chromophores are trace glycerides and were concentrated by silica column chromatography. The concentrated color bodies were hydrolyzed and analyzed as free acids or methyl esters by reversed-phase high-performance liquid chromatography with photodiode array and mass spectrometry detection, ^1H and ^{13}C nuclear magnetic resonance and infrared spectroscopies. Absorbance in deodorized oils is mostly from oxygenated C18 and C20 fatty acids with 1 to 4 double bonds. High-wavelength absorbance in AS-bleached oils is from conjugated pentane fatty acids that are not observed for ABE-bleached oils. Thus, both the bleaching agent and the deodorization treatment affect the distribution and concentration of such stable chromophores.

KEY WORDS: Bleaching, canola oil, conjugated polyunsaturated fatty acids, deodorization, electronic absorbance spectra, HPLC, mass spectra, pigments.

Silica hydrogel adsorbents are effective for the removal of phospholipids, soaps and associated trace metals from triglyceride oils (1). When silica hydrogels are acidified with strong mineral acids, such as sulfuric or phosphoric acid, they also remove chlorophyll pigments several times more effectively than acid-activated bleaching earth (ABE) (2–5). However, silica hydrogels acidified with sulfuric acid (AS) also suffer from disadvantages that prevent commercial-scale use (5). For example, AS adsorbents do not remove β-carotene effectively, and after AS-bleached oils are deodorized, Lovibond Tintometer red and yellow (R/Y) numbers are greater than those of ABE-bleached and deodorized oils. These stable R/Y colors do not arise from decomposition products of β-carotene (5).

Generally, color development in triglyceride oils during processing is associated with oxidation. Thus, Golumbic (6,7) demonstrated that oxidized tocopherols, particularly deep red tocoquinones, were responsible for color produced by autoxidation. King and Wharton (8) showed that oxidation caused formation of new pigments and stabilized existing pigments against adsorption. Color development in refined oils is associated with phenolic pigments, e.g., the characteristic yellow color of refined cottonseed oil (9) and palm oil (10) is from gossypol and related phenolic pigments, respectively. Color development in processed soybean oil has been attributed to unsaturated carbonyl groups (11), and a recent report (12) attributes fixed colors in rice bran oil to monoglycerides with oxidized fatty acids. The present study was undertaken to identify the chromophores responsible for color in deodorized oils, and particularly the nature of the differences between ABE- and AS-treated oils. The identification of such pigments is expected to aid in the design of improved chlorophyll adsorbents.

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EXPERIMENTAL PROCEDURES

Super-degummed canola oil from the 1991 crop was obtained from CSP Foods (now CanAmera Foods, Russell, Manitoba, Canada). The oil was further degummed at the laboratory scale by heating the oil to 40°C, adding 0.1 wt% phosphoric acid (85%), stirring the oil for 10 min, adding 1.0 wt% water, stirring for an additional 10 min, and centrifuging off the impurities. The trace element impurity levels were measured by inductively coupled plasma spectrophotometry. The ABE was Filtrol F160 (Englehard, Edison, NJ), which contained 18% volatile water (measured at 550°C). TriSyl® Silica (W.R. Grace & Co.-Conn., Columbia, MD) contained 65% volatile water. The acidified silicas were prepared simply by physically mixing concentrated sulfuric acid (96%, reagent-grade, J.T. Baker, Phillipsburg, NJ) with commercially available TriSyl® silica. AS6 contained 6 wt% sulfuric acid on a hydrous basis and AS10 contained 10 wt% sulfuric acid.

Solvents used (as received) were hexane [Baker analyzed high-performance liquid chromatography (HPLC)-grade, 97% *n*-hexane], ethyl acetate (Baker analyzed reagent-grade), 2-propanol (*i*PrOH, Baker analyzed HPLC-grade), acetonitrile (MeCN, Baker analyzed HPLC-grade), tetrahydrofuran (Baker analyzed HPLC-grade) and glacial acetic acid (Baker reagent-grade, 100.0% CH_3COOH). HPLC-grade 18 MΩ water was produced in a Milli-Q system (Millipore Corp., Milford, MA).

Bleachings were done at 100°C for 30 min with vigorous stirring at 10–20 mbar pressure. The ABE-bleached oil was prepared with 2.00 wt% F160 (anhydrous basis). The AS10-bleached oil was prepared by using 0.60 wt% AS10 (hydrous basis). Deodorizations were carried out at 250°C for 1 h at < 1 mbar with injection of 2 wt% HPLC-purity water. Water vapor was fed continuously over the course of the deodorization by means of a needle valve. Bleached oils are designated by "B," while bleached and deodorized oils are designated by "B/D."

Free fatty acids (FFA) were prepared by enzymatic hydrolysis with lipase (Sigma Type II, crude, from porcine pancreas; Sigma Chemical Co., St. Louis, MO) by reaction of 0.1 to 0.5 *g* fat with 0.5 *g* lipase at 37°C for 1 h in 50 mL water buffered at pH 7.4. A homogenizer was used to emulsify the mixture. After the reaction, the mixture was acidified to pH 3–4, cooled in an ice bath and filtered to isolate the precipitated fatty acids and lipase. The recovered solids were extracted with $CHCl_3$ in a Soxhlet extractor, and the aqueous filtrate was extracted twice with 50-mL portions of $CHCl_3$. The $CHCl_3$ extracts were then combined and evaporated to dryness. Fatty acid methyl esters (FAMEs) were prepared *via* AOCS Method Ce 2-66 (13). Free fatty acid titrations were performed according to AOCS Method Ca 5a-40 (13).

Ultraviolet and visible (UV/VIS) spectroscopic data for all oil samples were obtained with a Perkin-Elmer Lambda 3A spectrophotometer and 3600 Data Station (Palo Alto, CA). Samples were diluted in solvent and were run in either 0.1- or 1.0-cm cells with solvent in matched cells as reference. Tintometer data were obtained with a

Lovibond AF960 Tintometer (HF Scientific, Ft. Myers, FL). Infrared spectroscopy data were obtained with a Nicolet 205 spectrometer operated in the Fourier transform mode from capillary oil films between sodium chloride windows. High-resolution ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra for samples dissolved in $CDCl_3$ were obtained on an ACE 300 MHz Bruker NMR Spectrometer (Burlington, Ontario, Canada). Chemical shifts are reported vs. tetramethylsilane.

Gel permeation chromatography (GPC) was performed on an HP 1090 Liquid Chromatograph with a Phenonomex 3 Linear Phenogel 5μ column with radioimmunoassay detection. Chromatograms were obtained with THF as eluant at ambient temperature, essentially following AOCS Method Cd 22-91 (13).

Reversed-phase HPLC with photodiode array detection (HPLC/UV-VIS) was employed to determine the electronic spectra of individual fatty acids. All analytical HPLC separations were carried out by a Beckman 114M pump (Fullerton, CA) and 421 controller with a Waters 990 photodiode array detector (Milford, MA). FFA separation was carried out at ambient temperature on a 5-μ Supelcosil LC-8 column (25 × 0.46 cm) and C8 Supelcosil Supelguard precolumn (Supelco, Bellefonte, PA). Samples were prepared as 5% (wt/vol) solutions in hexane in amber vials. Each sample (10 μL) was injected isocratically at 1 mL/min with MeCN/THF/0.1% aq. H_3PO_4 (50.4:21.6:28.0, vol/vol/vol) as eluant. The FAME separation was carried out at ambient temperature on a 5-μ Supelcosil LC-18 column (25 × 0.46 cm) and C18 Supelcosil Supelguard precolumn (Supelco). Samples were prepared as 10% (wt/vol) solutions in hexane in amber vials. Each sample (25 μL) was injected isocratically at 1 mL/min with MeCN/*i*PrOH/water (58:25:17, vol/vol/vol) as eluant. Chromatograms and UV spectra of eluting compounds were collected between 200 and 400 nm with 5 nm spectral resolution. All polar compounds of interest eluted in less than 20 min under these conditions. Chromatograms were initially represented and compared as contour plots with retention time on the x-axis, wavelength on the y-axis and absorbance on the z-axis. Comparison of spectra was done after appropriate background subtraction on the photodiode array data system.

HPLC/mass spectrometry (MS) studies of the FAMEs were performed on a Finnigan TSQ 70 mass spectrometer (San Jose, CA) equipped with a thermospray liquid chromatography/MS interface by using discharge ionization. The repeller potential was set at zero volts to facilitate the determination of molecular weight. It was necessary to specifically monitor the molecular ions of the some of the analytes present at low levels to observe them by MS.

Normal-phase column chromatographic fractionations were performed on 40 g silica gel (Aldrich 70–230 mesh, 60 Å pore diameter, ~500 m^2/g surface area, 0.75 cm^3/g pore volume; Aldrich Chemical Co., Milwaukee, WI) in a 30-mm inner diameter column (~90-mm bed length). Thus, 5.00 g of oil was accurately weighed out and dissolved in 50-mL hexane, and this solution was loaded onto the column with gravity flow. The loaded column was then washed with 100 mL hexane, and the following elution sequence was used (fractions from this procedure are designated F1, F2, etc.): F1, 200 mL 99:1 (vol/vol) hexane/ethyl acetate; F2, 50 mL 95:5 (vol/vol) hexane/ethyl acetate; F3, 50 mL 95:5 (vol/vol) hexane/ethyl acetate; F4, 50 mL 95:5 (vol/vol) hexane/ethyl acetate; F5, 50 mL 95:5 (vol/vol) hexane/ethyl acetate; F6, 100 mL 80:20 (vol/vol) hexane/ethyl acetate; F7, 100 mL 80:20 (vol/vol) hexane/ethyl acetate; F8, 200 mL 95:5 (vol/vol) hexane/*i*PrOH; F9, 200 mL 70:29:1 (vol/vol/vol) hexane/*i*PrOH/acetic acid. The solvents were removed from the collected fractions *via* a nitrogen stream at ambient temperature, and the recovered fractions were weighed. The fractions were then redissolved in hexane for quantitative UV/VIS and qualitative thin-layer chromatography (TLC) analysis. No attempt was made to exclude atmospheric oxygen or light during chromatographic separations. The fractions were stored in a freezer (T < 0°C).

Normal-phase TLC plates were commercially precoated with silica gel containing a fluorescent indicator (Sigma Chemical, 250 μm layer, 5–17 μm particle size, 60 Å pore diameter). The plates were developed in 80:20 (vol/vol) hexane/ethyl acetate, and were visualized with UV radiation (254 nm) and by reaction with iodine.

RESULTS AND DISCUSSION

Oil quality data are given in Table 1. The starting oil was bleached to low levels of chlorophyll and phosphorus with ABE or AS10. The high sulfur content of the AS10 bleached oil is from sulfuric acid that leached into the oil during bleaching. After the bleach treatment, the AS10-treated oil was reddish-brown, primarily from the presence of β-carotene as has been demonstrated (5). After deodorization, the AS10-treated oil had much greater tintometer R/Y numbers than the ABE-treated oil, and the former had a visibly deeper yellow color than the latter. The presence of the low level of sulfate in the AS10 oil (which is largely removed during deodorization) does not influence deodorized R/Y numbers because sulfate-free AS-bleached oils also have high R/Y numbers after deodorization. The high R/Y numbers after deodorization could not be attributed to β-carotene decomposition products (5).

The starting oil had essentially no absorptivity (L/g•cm) in the range 250–340 nm, and at higher wavelengths, the absorptivity was from the presence of chlorophyll and β-carotene (data not shown). ABE- and AS10-bleached oils had substantial absorbance between 250 and 320 nm, and the absorption bands correspond to those of conjugated polyunsaturated fatty acids (PUFAs) produced by alkali isomerization (Table 2). The formation of conjugated PUFAs during bleaching is well recognized (19–24), and results from the acid-catalyzed decomposition of fatty acid hydroperoxides. For example, the formation of conjugated tetraene is the product of C18:3 fatty acid hydroperoxide decomposition.

The absorptivity between 220 to 400 nm was greater for the AS10 B oil than for the ABE B oil (data not shown). Furthermore, the conjugated pentane absorption at 345 nm was present for the AS10 B oil but was much lower for the ABE B oil. The upper limit for conjugated pentane concentration was 100 ppm. Therefore, the levels and distribution of conjugated PUFAs are strongly affected by the adsorbent treatment.

The UV spectra of the deodorized oils were relatively featureless, and the vibrational structure assigned to conjugated triene and tetraene for bleached oils was much less distinct (data not shown). Furthermore, the band for conjugated pentane was absent for the AS10 B/D oil. The

TABLE 1

Quality of Superdegummed Canola Oils After Bleaching and Deodorization

Description	Chlorophyll A (ppm)	Tintometer numbers Red	Yellow	Phosphorus (ppm)	Iron (ppm)	Sulfur (ppm)	Free fatty acids (wt%)
Initial oil	16.6	—	—	5.5	0.0	3.7	0.36
ABE bleached[a]	0.04	0.1	0.7	0.0	0.0	3.3	0.28
Deodorized[b]	0.01	0.6	4.9	0.0	0.0	2.9	0.00
AS10 bleached[a]	0.08	4.5	>70	2.7	0.0	139.5	0.65
Deodorized[b]	0.00	12	>70	5.9	0.0	20.0	0.06

[a]Tintometer (HF Scientific, Ft. Myers, FL) red/yellow (R/Y) numbers in 1.0-cm cells. ABE = activated bleaching earth; AS10 = 10 wt% sulfuric acid on TriSyl® silica (W.R. Grace & Co.-Conn., Columbia, MD).
[b]Tintometer R/Y numbers in 5.25″ cells.

TABLE 2

Absorption Band Maxima for Processed Canola Oils and Reference Compounds

Description	Band maxima (nm)	Comments[b]
ABE bleached[a]	345, 315, 300, 278, 268, 257	C3=; C4; C5=
AS10 bleached[a]	345, 326, 315, 300, 278, 268, 257	C3=; C4=; C5=
ABE bleached/deodorized	277	
AS10 bleached/deodorized	278, 268, 257	C3=
c-9,*t*-11, *t*-13-Octadecatriene (C3=)	259, 271, 279	Reference 14
t-9, *t*-11, *t*-13-Octadecatriene (C3=)	258, 268, 279	Reference 14
Octadecatetraene (β-parinarate) (C4=)	288, 301, 315	References 15,16
Eicosatetraene (arachidonate) (C4=)	301, 315	References 16–18
Docosapentaene (C5=)	315, 328, 346	References 16,18
Eicosapentate (C5=)	315, 328, 346	References 16,18

[a]See Table 1 for abbreviations and company sources and addresses.
[b]Nomenclature: C3= represents conjugated triene, etc.

spectra also revealed that absorptivity in the 220–240 nm range increased dramatically during deodorization. Thus, reaction of the conjugated PUFAs occurred during deodorization.

Between 220 and 400 nm, the absorptivity for the AS10 B/D oil was greater than that for the ABE B/D oil (data not shown). Above 400 nm, the absorptivity for both oils was negligible. However, UV band tails that absorb in the visible region were more pronounced for the AS10 B/D oil than for the ABE B/D oil. The Tintometer R/Y numbers thus relate to this featureless tailing into the visible (>350 nm) from UV absorption bands.

Crude fractionation of an AS10 B/D oil was performed to demonstrate that the chromophores were glyceride-based. The most highly colored fractions were thus analyzed by ^{1}H and ^{13}C NMR and infrared (IR) spectroscopies, and the FAMEs were analyzed by GC (data not shown). The fractions were predominantly composed of unsaturated triglycerides and partial glycerides and/or hydroxylated fatty acids. No aromatic resonances were observed, and there was no evidence for aldehyde or ketone functionalities. The *trans*-double bonds were identified by IR and GC in deodorized oils as has been reported (25–27).

The normal-phase column fractionation procedure was used to concentrate the pigments of the four processed samples of Table 1. The mass percentages of the various fractions are given in Table 3. There were no significant differences between the silica gel TLC plates of the various

TABLE 3

Mass Percentages of Various Oil Fractions by Normal-Phase Column Fractionation[a]

Oil description	F1	F2	F3	F4	F5	F6	F7	F8	F9
Starting oil	<0.1	0.8	38.4	49.9	5.8	2.9	1.3	0.2	0.6
ABE bleached[b]	<0.1	<0.1	42.8	49.3	4.2	2.0	1.2	0.1	0.4
AS10 bleached[b]	<0.1	<0.1	35.9	54.4	5.0	2.2	1.5	0.2	0.7
ABE bleached/deodorized	0.1	<0.1	40.5	50.4	5.2	2.5	1.2	0.2	<0.1
AS10 bleached/deodorized	0.2	0.9	33.1	51.6	8.8	2.9	1.6	0.3	<0.1
ABE bleached/deodorized	<0.1	0.1	38.7	50.3	6.4	2.8	1.5	0.1	<0.1

[a]F1 designates Fraction #1, etc.
[b]See Table 1 for abbreviations.

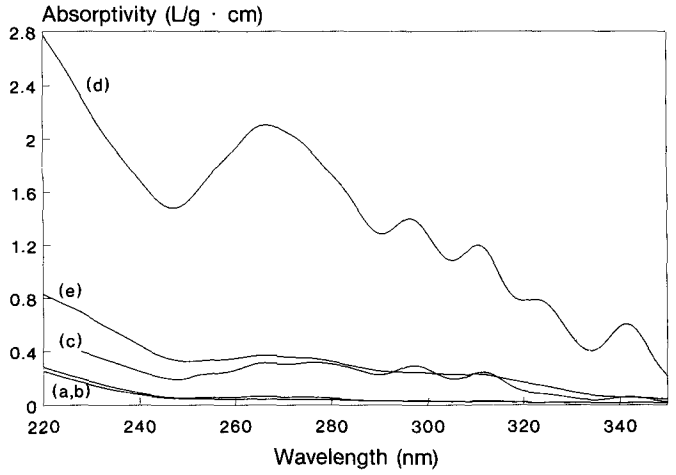

FIG. 1. Ultraviolet/visible absorbance spectra for chromatographic fractions from 10 wt% sulfuric acid on TriSyl® silica (W.R. Grace & Co-Conn., Columbia, MD) bleached oil. F3, (a); F4, (b); F5, (c); F6, (d); F7, (e).

oils. TLC results for the fractions were as follows: F2 through F5 for all of the samples showed only one spot, with $R_f > 0.90$; F6 had bands from $R_f = 0.37$ to 0.90, and F7 showed bands with $0.31 < R_f < 0.65$. The R_f values for the partial glycerides 1,3-dipalmitin and 1-monopalmitin were 0.48 and 0.06, respectively, under the same conditions.

The F6 fractions were visibly the most highly colored fractions, and had the highest absorptivity between 220 and 400 nm as shown for the AS10 B oil. (Fig. 1: The UV/VIS data in this figure are shifted 3 nm to lower wavelengths because of a wavelength calibration error.) The mass-weighted absorptivities (mass fraction·absorptivity) calculated at 345 nm for all of the oils revealed that the F6 fractions represented the following percentages of absorbance for the bulk oils: starting oil, 22%; ABE B oil, 42%; AS10 B oil, 66%; ABE B/D oil, 29%; AS10 B/D oil, 69%.

To further demonstrate that fractions with $R < 0.90$ caused the high Tintometer numbers for the AS B/D oils, an AS6 B oil was fractionated to yield oil with $R_f > 0.90$, which by GPC analysis was pure triglyceride. The Tintometer R/Y numbers for this oil were 11/> 70 (5.25″ cell) prior to deodorization, and were 0.3/1.5 for the colorless oil after deodorization, confirming that the later-eluting fractions are responsible for most of the color of AS B/D oils.

GPC was used to investigate the molecular weight distributions of the various fractions. All of the F6 fractions for both the B and B/D oils contained triglyceride dimers. For both the ABE B and AS10 B oils, the percentage of dimer increased during deodorization, so that after deodorization the levels were comparable. The levels of dimer calculated for the bulk oils are: ABE B, 0.13 wt%; AS10 B, 0.29 wt%; ABE B/D, 0.52 wt%; AS10 B/D, 0.58 wt%. These data suggest that triglyceride dimerization occurs during both bleaching and deodorization.

As demonstrated above, a significant portion of the chromophores were concentrated in the F6 fractions. These fractions were then hydrolyzed enzymatically to prepare the corresponding FFAs, or were saponified/esterified to prepare the FAMEs. To determine the effect of hydrolysis and esterification on the chromophores, electronic spectra were quantitatively compared for the AS10 B sample, as well as for commercial trilinolenin, before and after reaction. Some differences were noted in the absorptivities of the bands after esterification, but the peak maxima and band shapes were essentially identical. By contrast, the spectra for samples prepared by enzymatic hydrolysis were unchanged. We believe that some geometric isomerization of conjugated PUFAs occurs during the esterification procedure, and that allylic alcohols will be dehydrated under these conditions. The esterification procedure does not catalyze positional or geometric isomerization of unconjugated PUFAs (25), but double-bond conjugation may stabilize carbenium ion intermediates enough to allow isomerization of conjugated PUFAs. Because analyses described below are neither geometric nor positional isomer-specific, and because the FAMEs were treated identically, it is concluded that comparisons of the esterified samples are valid. A recent report (28) details this reaction of conjugated PUFAs during methylation with boron trifluoride.

Reversed-phase HPLC separation with UV/VIS photodiode array detection of F6 acids is a very sensitive method for determination of the electronic spectra of the individual fatty acids, and a method similar to that reported here was recently published (29). Data for the FAMEs are shown in Table 4 and Figures 2 and 3. FAMEs with the highest wavelength absorbance eluted with retention times (R_t) less than 12 min, while unconjugated methyl linolenate eluted at 13.2 min and unconjugated methyl linoleate eluted at 20.0 min.

The chromatograms of bleached oils were similar, except that the maximum absorbance in the range 330 to 350 nm for AS10 B was substantially greater than that of ABE B for most of the analytes (Table 4 and Fig. 2). Analytes with absorbance in this wavelength range, which were identified based on their electronic spectra, included three separate conjugated pentane FAMEs with R_t at 6.44, 8.79 and 9.04 min, and seven conjugated tetraene FAMEs with R_t at 5.28, 5.50, 5.86, 7.84, 8.18, 10.99 and 12.20 min. Because the band maxima of conjugated PUFAs are geometric and positional isomer-sensitive, it is interesting to note differences in the absorption maxima reported in Table 4. These data confirm that the levels of conjugated pentaenes in the AS10 B oil were much greater than those for the ABE B oil, as was observed in the electronic spectra for the bulk oils. Analytes with only a single, broad peak in their optical spectra were noted with R_t of 3.49, 4.20, 4.36, 4.99, 5.10, 5.67, 5.94, 6.59, 7.02 and 13.05 min. Peak maxima for the main members of this class of chromophores were at 275, 305, 317 and 350 nm.

The F6 fraction from the AS10 B oil was also analyzed after enzymatic hydrolysis by HPLC/UV-VIS. The chromatograms for this sample displayed fewer features than for the analysis on the FAMEs. The predominant species observed were as follows: Two conjugated pentaenes were observed at 4.3 and 5.1 min; one conjugated tetraene was observed at 5.8 min; one conjugated triene was observed at 6.9 min and three broad bands, each with a single maximum at 277 nm, were observed at 4.0, 4.4 and 4.5 min. Comparison of these data to those reported above for the AS10 B FAMEs leads to the conclusion that the

TABLE 4

Early-Eluting Bands from High-Performance Liquid Chromatography/Ultraviolet–Visible Spectroscopy

Peak number	Retention time (min)	Description	Wavelength[a] maxima (nm)	Absorbance at maximum[b] AS10 B	ABE B	AS10 B/D	ABE B/D
1	3.49	b[c]	317	0.18	0.05	0.11	0.12
2	4.20	b	320	m[c]	m	m	m
3	4.36	b	305	0.42	0.19	0.27	0.19
4	4.99	b	350	0.21	0.06	N/O	N/O
5	5.10	b	275	0.28	0.21	1.08	0.01
6	5.28	ct	290,301,315	0.42	N/O	N/O	N/O
7	5.50	ct	289,302,316	m	m	m	m
8	5.67	b	317	m	m	m	m
9	5.86	ct	290,303,317	0.32	N/O	N/O	N/O
10	5.94	b	318	m	m	m	m
11	6.44	cp	315,330,348	0.16	N/O	N/O	N/O
12	6.59	b	275	0.80	0.68	0.71	0.81
13	7.02	b	275	0.75	0.63	0.64	0.76
14	7.84	ct	291,303,317	0.12	0.08	N/O	N/O
15	8.18	ctr,ct	268,279,302,315	0.09	0.06	0.08	0.04
16	8.79	cp	302,316,332,350	0.18	0.04	0.05	N/O
17	9.04	cp	302,314,329,347	0.15	0.04	0.06	N/O
18	10.99	ct	291,304,317	0.28	0.27	0.16	0.14
19	12.20	ct	288,300,315	0.19	0.14	0.13	N/O
20	13.05	b	245	N/O	N/O	0.55	0.77
21	16.04	ctr	260,268,280	0.09	0.10	0.14	0.10
22	17.74	ctr	260,270,280	0.10	0.16	0.17	0.15

[a]Peak with maximum absorbance is underlined.
[b]Absorbance corrected for sample size. See Table 1 for abbreviations.
[c]Key: b = broad, single maximum; ctr = conjugated triene; ct = conjugated tetraene; cp = conjugated pentaene; N/O = not observed, m = minor.

esterification reaction caused some reaction of the conjugated PUFAs.

For the F6 FAMEs, maximum absorbance in the range 295 to 315 nm (Fig. 3) is substantially greater for the AS10 B/D oil than for the ABE B/D oil, which demonstrates that higher levels of the chromophores are present in the former case. For the AS10 B/D oil, conjugated pentaenes are observed with R_t = 9.04 min, and conjugated tetraenes are observed with R_t = 10.99 min and 12.20 min. In contrast, the conjugated tetraene at 10.99 min is the only polyene with more than three double bonds that is clearly identifiable for the ABE B/D oil. The predominant species that have absorbance above 300 nm (R_t = 3.49, 4.10, 4.36, 5.67, 5.94 min) display spectra with a single peak in the range 275 to 320 nm, as indicated in Table 4. Thus, most of the conjugated tetraenes and pentaenes present before deodorization undergo reaction during deodorization. A notable difference in the deodorized oils is in the level of the analyte at 5.10 min, which is the predominant component for the AS10 B/D oil, and is only a trace component for the ABE B/D oil.

HPLC/MS was performed on the F6 FAMEs to help identify the nature of the analytes with broad electronic spectra. The predominant components identified in all of these fractions were unconjugated C18:3 and C18:2 esters that had retention times near 14 and 22 min, respectively, in good agreement with the respective R_t of 13.2 and 20.0 min in the HPLC/UV-VIS experiments. However, low levels of other PUFAs were also detected, which eluted in less than 12 min. Thus, for the bleached oils, the following esters were detected (where O-C18:1 designates a C18 ester with one degree of unsaturation and one additional oxygen): C18:1, C18:2, C18:3, C18:4, C18:5; O-C18:1, O-C18:2, O-C18:3, O-C18:4, O-C18:5, O-C20:0, O-C20:1, O-C20:2 and O-C20:3. For the deodorized oils, the following esters were detected: O-C18:2, O-C18:3, O-C18:4, O-C18:5, O-C20:1, and O-C20:2. These assignments were made on the basis of the molecular weights of the analytes as well as the characteristic fragmentation pattern for oxygenated methyl esters (loss of one methanol and two water molecules from the protonated molecular ions). The chromophores that did not display vibrational structure in the HPLC/UV-VIS experiments are thus oxygenated, conjugated PUFAs. Based on pure compound electronic spectral data, analytes in Table 4 with maxima near 275 nm (5,12 and 13) are possibly hydroxy-triene, or oxo-diene fatty acids. To differentiate between these two possibilities, ^1H and ^{13}C NMR spectra and IR spectra were obtained. Small peaks at 3.3 and 3.4 ppm in the ^1H spectra were consistent with alcohols, but no carbonyl groups were observed. IR data confirmed the presence of hydroxyl because a small band was observed at 3466.7 cm^{-1}, and no other features indicative of keto-carbonyls (30) were found. Thus, oxygenated, unsaturated FAMEs are thought to be predominantly in the hydroxyl form.

Because conjugated tetraenes and pentaenes were present in the bleached oils prior to deodorization but were largely absent after deodorization, it is plausible that such highly conjugated PUFAs undergo hydration reactions during deodorization to form hydroxylated fatty acids.

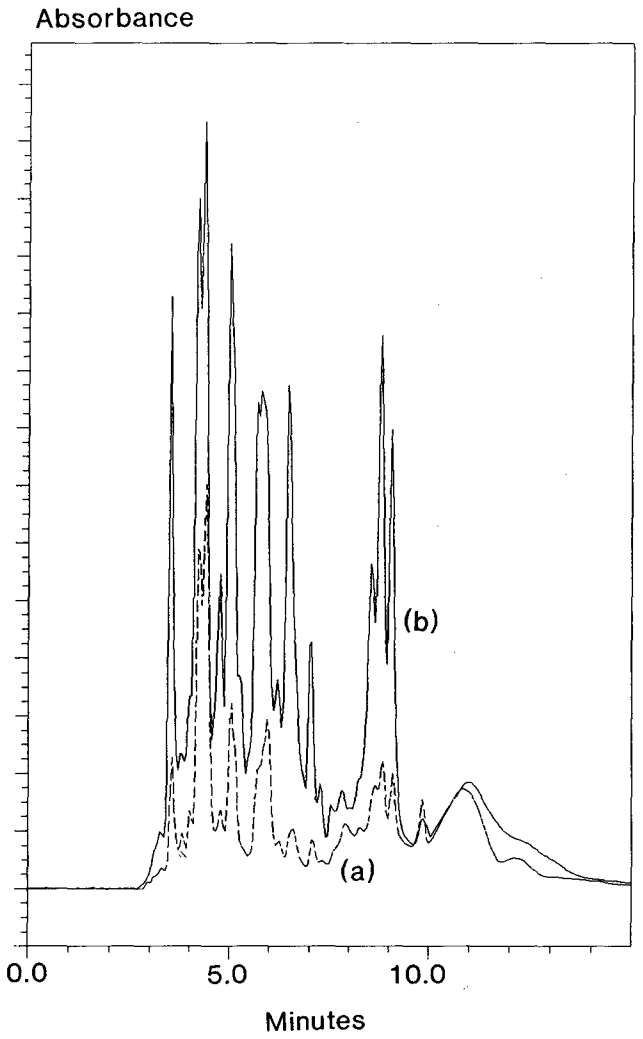

FIG. 2. High-performance liquid chromatography/ultraviolet–visible chromatogram for esterified fractions 6 from activated bleaching earth bleached oil (a) and from 10 wt% sulfuric acid on TriSyl® silica (see Fig. 1 for company source) bleached oil (b). Ordinate represents maximum absorbance within the range 330 to 350 nm.

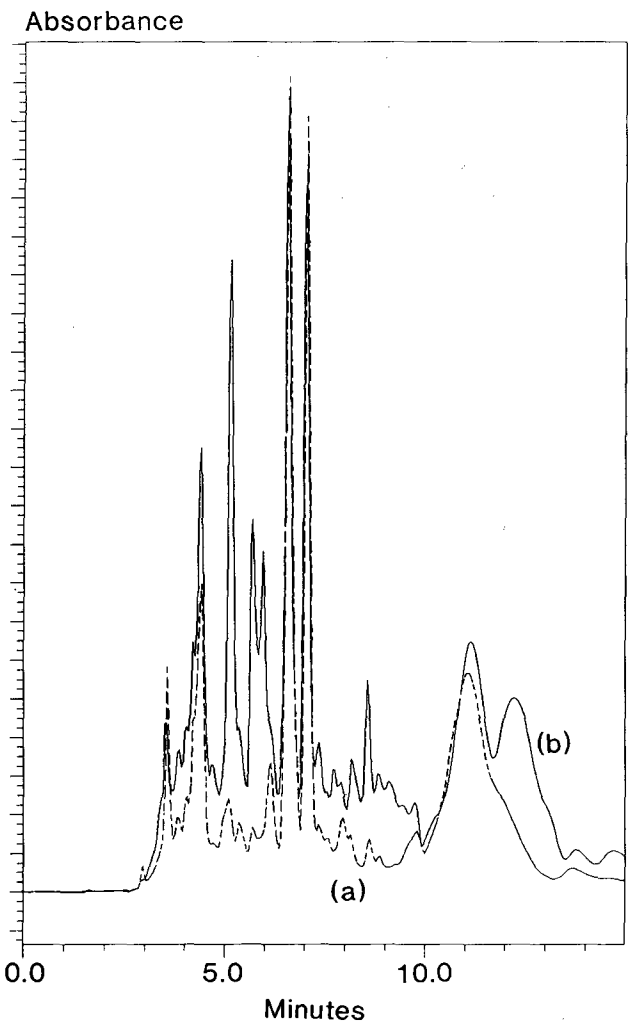

FIG. 3. High-performance liquid chromatography/ultraviolet–visible chromatogram for esterified fractions 6 from activated bleaching earth bleached and deodorized oil (a) and from 10 wt% sulfuric acid on TriSyl® silica (see Fig. 1 for company source) bleached and deodorized oil (b). Ordinate represents maximum absorbance within the range 295 to 315 nm.

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