SHORT COMMUNICATION

Characterization of Wax Sediments in Refined Canola Oils[1]

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Turbidity components in refined canola oils were collected by filtration at 4 and 20°C. Major components (thin-layer chromatography) at both temperatures were wax esters (WE), hydrocarbons (HC) and triacylglycerols (TG) while free fatty acids (FFA) and fatty alcohols (FAL) were found in minor amounts at 4°C. WE had carbon numbers of 40 to 56 (made up of combinations of C16 to C30 alcohols and C16 to C28 fatty acids). HC were mostly C29 and C31 with lesser amounts of C24, C28 and C32. TG, compared to the corresponding liquid oil, still contained C18:1 as the major component, but had less C18:2 and particularly less C18:3 and at least two times higher levels of saturated fatty acids. The FFA were mainly long-chain C22:0, C24:0 and C20:0 (in order of amount). FAL were mainly long-chain C26:0, C28:0 and C30:0 but ranged from C16:0 to C32:0.

KEY WORDS: Alcohols, canola, fatty acids, gas-liquid chromatography, hydrocarbons, thin-layer chromatography, triacylglycerols, turbidity, wax, wax esters.

Canola oil is considered to be an excellent salad oil because of its advantageous fatty acid composition and its clarity when stored at low temperature. Over the past 10 years, however, the canola industry has seen the occasional appearance of haze or sediment in salad oil during storage in both refrigerator and store shelves. Recently, the haze problem in canola oil has become more important because of the demand for high-clarity oils in the United States market. The nature of this sediment was not well understood. Other salad oils, such as sunflower seed oil, crystallize at low temperature, making necessary the removal of the crystals by a process known as winterization (1), but canola and rapeseed oils have not traditionally required winterization.

Studies on the haze materials in sunflower seed oil showed that the oil components causing turbidity were high-molecular weight compounds, such as saturated triacylglycerols, wax esters, free fatty acids and, to a lesser extent, hydrocarbons and fatty alcohols, etc. (2–4). Little is known about the sediment composition of canola oil, however, and the main objective of the present work was to characterize the wax sediments.

MATERIALS AND METHODS

Canola oil. Refined and bleached canola oils that had developed sediment were obtained from Western Canadian canola processors.

Collection of sediment. The sediment, observed as a cloudy or haze layer, usually near the bottom of the bottle, was collected by vacuum filtration at 4 or 20°C through Hyflo Super-cel filter aid (Fisher Scientific, Ontario, Canada). The filtrate was rinsed with cold (*ca.* 4°C) hexane to remove residual liquid oil, and the remaining sediment was eluted with hot (*ca.* 40°C) toluene. The sediment was obtained after distillation of the toluene in a rotary evaporator. Melting points were determined with a Mettler (Cincinnati, OH) FP5 melting point apparatus.

Separation of sediment components. Sediment components were separated by thin-layer chromatography (TLC) with toluene/chloroform (7:3) as the developing solvent. Analytical separation was carried out on 250-μM plates (Whatman silica gel LK5D, Maidstone, England). Preparative separations were carried out on 1000-μM plates (Whatman silica gel LK6). The wax ester (WE), triacylglycerol (TG), hydrocarbon (HC), free fatty acid (FFA) and fatty alcohol (FAL) bands were removed for subsequent analyses by gas-liquid chromatography (GLC) by scraping, and, after soaking the scraped silica gel with chloroform, by eluting with chloroform.

FFAs. The FFAs were converted to methyl esters by acid-catalyzed esterification (5). The composition of the mixture was determined by GLC with conditions: column—15 M by 0.32 MM i.d. open tubular fused silica with a 0.5 μM Supelcowax 10 coating (Bellefonte, PA); temperature program—1 min at 210°C, then to 240°C at 10°C/min and hold 5 min; injection temperature—250°C; carrier gas—hydrogen 75 cm/s. The equivalent chain-lengths (ECNs) of unknown fatty acids were determined from a plot of log-adjusted retention time *vs.* ECN for an isothermal analysis at 230°C. Calibration samples were available only up to 24, and estimates beyond ECN 24 are only tentative.

FALs. Fatty alcohols were separated without derivatization by a similar GLC procedure as for fatty acid methyl esters, with final holding times extended to 20 min. ECNs were determined as above.

TGs. The intact triacylglycerol fraction was separated by GLC: column—30 M by 0.32 MM column coated with 0.25 mM SPD-5; temperature program—240 to 320°C at 40°C/min followed by 2°C/min to 360°C; injection temperature—240°C (on column); carrier gas—helium 35 cm/s. Methyl esters of the TG fraction were prepared by alkali-catalyzed methanolysis (6) and were determined as above (FALs).

WEs. Intact WEs were analyzed by the same procedure as for TGs. The WE fraction was subsequently hydrolyzed by alkali-catalyzed saponification (5). Component FAs and FALs were determined as above.

HCs. The HC fraction was separated by GLC procedure as for FALs.

RESULTS AND DISCUSSION

Amount of sediment in oils. Different amounts of sediments were obtained depending on the source of oils and collection conditions. In an earlier study in our laboratory, samples of oil were obtained that had about 10 times more sediment than was found in the samples used in this work (7).

The amount of wax fraction from oil collected at room temperature was much less (approximately one-seventh) than that from oils collected at cold temperature (Table 1). It was subsequently found that wax sediment could

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TABLE 1

Sediment Collection from Oils

Oil	A		B	C
Collection temperature (°C)	20	4	4	4
Sediment (mg/1000 mL)	73	10	67	37
Melting point (°C)	70–80	76–79	59–76	74–79

not be eliminated completely from the oil even after two dewaxing treatments at low temperature, although the treated oil might remain clear for up to two months.

Identification of wax components by TLC. The melting points of wax sediments from three different oils were determined, one had a melting duration from 59 to 76°C, and the others from 70 to 80°C and 74 to 79°C.

Five bands were observed after TLC of the wax fraction collected at cold temperature (4°C), and four bands for that collected at room temperature. The identity of the bands was determined by comparison with authentic reference materials; the band not present in the room temperature fraction was FAL. Major components in both cold- and room temperature-filtered sediments appeared to be WEs, followed by TGs and HCs. The sediments also contained minor amounts of FFAs and, for cold temperature sediment only, FALs.

In the earlier study (7), where more sediments were collected, TGs were found to be the major component of the sediment.

Individual WE, TG, HC, FFA and FAL components were obtained by preparative TLC from wax sediment isolated at 4°C. Each component was re-chromatographed by analytical TLC to examine purity. Both TG and WE components were always contaminated by HC.

Composition of TGs. The TGs from the sediment ranged in ECNs from C48 to C60 (Fig. 1A). The fatty acid composition of the TG showed that C18:1 was still the major component (about 46%), but the amounts of C18:2 and C18:3 were considerably lower and the saturated acids were about four times higher than those in related liquid oil or typical canola oil (Table 2). This composition corresponds fairly closely to the fatty acid composition reported for TGs isolated at room temperature from the previous study (7).

Composition of WEs. WEs had ECNs ranging from 36 to 56 with C36, C44, C46 and C48 being most predominant (Fig. 1B). The saponified fractions showed the waxes to be made up of a combination of long-chain FAs and FALs. FAs ranged from C16 to C32 in chainlength (Fig. 2A). FALs had chainlengths from C16 to C30 (Fig. 2B). This composition pattern is similar to the reported composition of sunflower oil wax, which is a mixture of FAs from C20 to C28 and FALs from C22 to C30 (1). The predominance of wax esters in the sediment suggests that, as with sunflower oil, these are the causative agent in sediment formation. Further investigation is required to determine the conditions under which sedimentation is initiated and whether there are varietal influences in the amounts of esters in the surface wax of canola seeds.

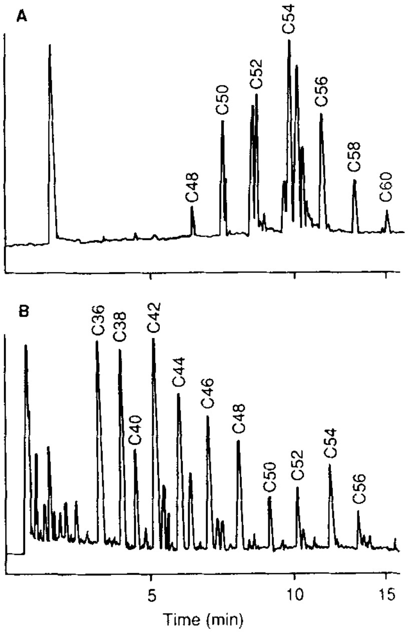

FIG. 1. Gas-liquid chromatogram of triacylglycerol (A) and wax ester (B) fractions isolated from sediment collected at room temperature.

TABLE 2

Comparison of Fatty Acid Composition Between Oil and Triacylglycerol Fraction from Sediment

	Triacylglycerol in	
Fatty acid (area %)	Sediment[a]	Oil[b]
C16:0	4.1	3.7
C18:0	4.5	1.7
C18:1	46.3	64.4
C18:2	13.5	18.4
C18:3	4.4	9.2
C20:0	4.5	0.7
C20:1	1.4	1.5
C22:0	5.3	0.3
C22:1	0.8	0.0
C24:0	4.7	0.2
C26:0	1.3	0.0
C28:0	2.2	0.0
Total saturates	26.6	6.6

[a] Isolated from fraction collected at 4°C.
[b] Oil from which sediment fraction was collected.

Composition of HCs. By comparison with the chromatogram of C24, C28, C32 and C36 standard paraffin mixture, major HC constituents separated from the wax sediment were C29 and C31 (about 80%) while C24, C28 and C32 showed in trace amounts. This is in agreement with an earlier report on HCs isolated from the surface of *Brassica napus* seeds, where nonacosane (C29) was the predominant component (8).

Composition of nonesterified FALs. Comparison with authentic reference materials of saturated FALs suggested that the nonesterified FAL fraction was made up of predominantly long-chain saturated alcohols C26:0, C28:0 and C30:0 (Fig. 2C). The nonesterified alcohols contained a much smaller proportion of shorter-chained alcohols

TABLE 3

Major Components of Nonesterified Fatty Acids Isolated from Sediment Collected at 4°C

Major components	C16:0	C18:0	C18:1	C20:0	C22:0	C24:0	Others
Area (%)	3.68	4.32	8.62	14.53	35.62	29.77	3.46

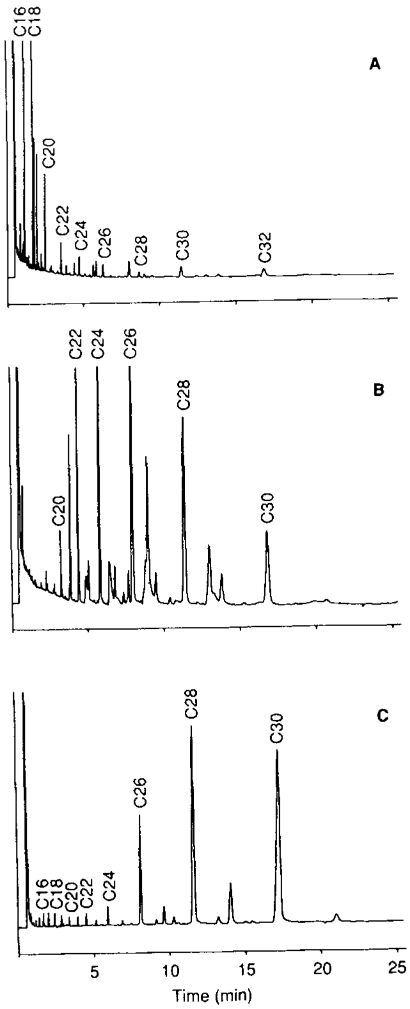

FIG. 2. Gas-liquid chromatogram of methyl esters of fatty acids (A) and alcohols (B) resulted from hydrolysis of wax esters, and nonesterified alcohols (C) from sediment collected at 4°C.

than were esterified in the WEs, but shorter-chained alcohols could have been lost in processing or in the analytical work-up, especially TLC.

Composition of nonesterified FAs. Nonesterified FAs from wax sediment were mostly saturated acids, especially the long-chain saturated C22:0, C24:0 and C20:0 in order of amount, and they made up approximately 80% of the total FA content (Table 3). As with the nonesterified alcohols, it is possible that some of the shorter-chained acids were lost during processing or in the analytical work-up.

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