Formation and Partial Characterization of Canola Oil Sediment[1]

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The occasional development of a haze in canola oil represents a problem to the quality and acceptability of this oil. The present study examined the formation of sediment in bottled canola oil during storage at 2, 6 and 12°C over a 4-d period. Oils stored at 2°C showed the highest rate of sediment formation, followed by storage at 6°C. Removal of sediment from canola oil prior to storage by cold precipitation and filtration did not eliminate this phenomenon, which still developed rapidly at 2°C. Chemical composition and thermal properties of canola oil sediment were compared to sediment obtained from commercial winterization of this oil. The thermal properties of the purified winterization sediment (melting temperature, 74.9°C) closely resembled those of the sediment from bottled canola oil. Saponification of both sediments yielded large amounts of long-chain fatty acids and alcohols, which were identified by gas chromatography-mass spectrometry. Sediment from commercial winterization contained higher amounts of fatty acids and alcohols with more than 24 carbon atoms in the chain.

KEY WORDS: Alcohols, canola oil, fatty acids, formation, identification, sedimentation, thermal property.

Some vegetable oils form a sediment or a cloudy haze during storage at room temperature. Canola oil usually passes the cold test and does not form a cloudy haze (1). Other oils, however, such as sunflower oil, form turbid sediments when stored at low temperatures and require winterization to remove it. Among the components implicated in turbidity formation are saturated triglycerides, waxes, free fatty acids, and to a lesser degree, hydrocarbons, sterols and their esters, and fatty alcohols (2–4). These components are present in refined, bleached and deodorized (RBD) oils in ppm and some in ppb concentrations, making their removal from oil extremely difficult (3,5,6). The components causing cloudy haze can also complex with nonhydratable phospholipids, which explains why phospholipids interfere with the winterization of sunflower oil (7,8).

Over the past few years, canola processors have occasionally observed a cloudy sediment after storage of the RBD oil, even at room temperature. The nature of this cloudiness remains unknown, and its occurrence cannot be predicted. The sediment formation in vegetable oils is generally attributed to waxy components and/or uncommon triglycerides, particularly those rich in saturated fatty acids (9). The problem of cloudiness is particularly prevalent in new hybrids of sunflower seeds, which contain up to five times more waxy components compared with normal varieties (10). New low-erucic acid and low-glucosinolate hybrids of rapeseed, referred to as canola, may also contain some of these minor constituents. This study examines the cause of sediment formation in canola oil and reports on the partial characterization of the sediments.

MATERIALS AND METHODS

Samples and chemicals. Commercial RBD canola oil samples, with and without sediment formation characteristics, were obtained from a Canadian processor. Both batches of these oils were processed by the same processor, produced from different batches of canola seeds and were not different as measured by standard methods of quality control. Both oils were packed commercially in plastic bottles.

All solvents used were chromatographic grade and were purchased from Burdick & Jackson (Baxter Diagnostics, Winnipeg, Canada). Fatty acid and alcohol standards were obtained from Nu-Chek-Prep (Elysian, MN).

Sample preparation for sedimentation kinetic study. To establish the rate of cloudy sediment formation, three bottles from the same batch of commercial canola oil, with a tendency to form a sediment, were randomly selected. These oils were heated to 75°C in a water bath to melt all crystals and then held for 2 h at this temperature. The oils were then stored at 2, 6 and 12°C for four days, and turbidity was monitored daily by measuring transmittance at 600 nm. The control was a canola oil sample not exhibiting sediment formation, which was treated in an identical manner and stored under the same conditions.

To examine the effect of sediment on turbidity formation, canola oil was stored at 0°C until sediment accumulated at the bottom of the bottle. The visible sediment was removed by centrifugation at 8000 × *g* for 60 min under refrigeration, followed by filtration at 2°C. The clear oil was then subjected to the same storage regime described previously.

Isolation and characterization of sediment. The sediments used in this study were isolated from canola oil, as described previously, as well as from a filter cake collected during commercial winterization of this oil. The filter cake was double extracted with boiling chloroform, and the solvent from the combined extracts was removed with a rotary evaporator. The oil was then stored at 2°C until a sediment appeared. This sediment was then removed by cold centrifugation and filtration, as described previously. The isolated sediments were washed with cold (0°C) petroleum ether to remove residual oil. The sediments used in this study were designated COS and IWS for commercial oil sediment and industrial winterization sediment, respectively.

Differential scanning calorimetry (DSC). The melting behavior of COS and IWS was examined with a DuPont 9900 (Wilmington, DE) thermal analyzer equipped with a 910 DSC pressure cell. Small amounts of sediment (3–4 mg dry solid fat) were hermetically sealed in aluminum DSC pans and heated from 5 to 140°C at 10°C/min with an empty pan as an inert reference. The DSC instrument was calibrated with indium while all other experimental conditions and data analyses were essentially those described by Biliaderis *et al.* (11).

Derivatization of fatty acids and alcohols. Washed sediments were saponified with 1N KOH in methanol at 80°C for 4 h in screw-top tubes. Following saponification, water was added, and the solution was extracted four times with 5 mL diethyl ether. The combined extracts were

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evaporated to dryness and acetylated with acetic anhydride in pyridine (1:2, vol/vol) for 2 h at 100°C. After removal of the unsaponifiables, the liquid was acidified with 2N HCl to pH 1.0, and the free fatty acids were extracted four times with 5 mL diethyl ether. Extracts were then combined and evaporated to dryness. Fatty acids were esterified with methanol-HCl at 80°C for 2 h.

During direct methylation of the sediments, additional sediment formed, which was isolated, washed twice with methanol and characterized by DSC and gas chromatography-mass spectrometry (GC-MS) after acetylation.

GC-MS. Acetates and esters were separated and identified by GC-MS. GC was performed on a Perkin-Elmer model 8500 (Norwalk, CT) gas chromatograph, while identification of compounds was carried out using a Finnigan Ion Trap Detector (San Jose, CA). Sediment components were separated on a capillary column (60 m × 0.25 mm i.d.), coated with DB-23 (J&W, Folsom, CA). Column temperature was programmed from 165 to 235°C at a rate of 2°C/min. The lower and upper temperatures were held for 2 and 30 min, respectively. For quantitation, correction factors were used that were obtained from running appropriate standards and from those published by Ackman and Sipos (12).

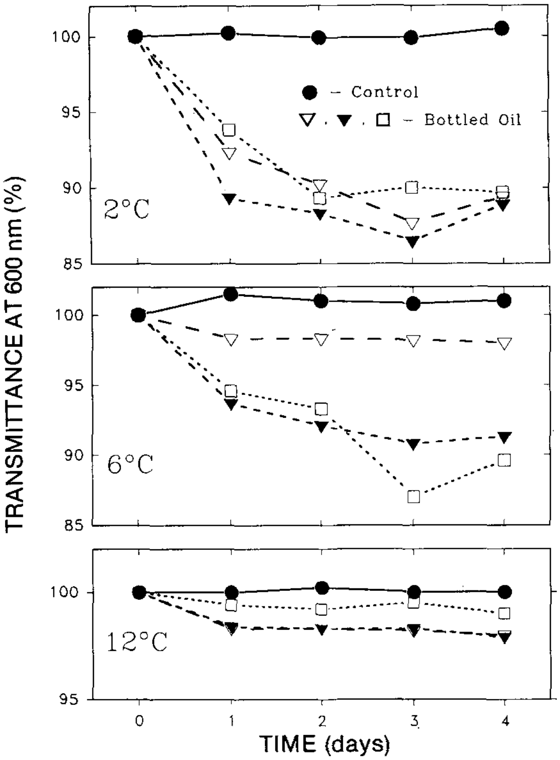

FIG. 1. Sediment formation during storage of canola oils in commercial plastic bottles at different temperatures.

RESULTS AND DISCUSSION

Rate of sedimentation formation. All oils stored at 2°C formed sediments at similar rates (Fig. 1). Oil samples stored at 6°C, the temperature used in industrial winterization, developed cloudy sediments at slower rates and were different for different bottles (Fig. 1). On the three bottles of oil used, from this same batch of canola oil but packed in different plastic bottles, one sample exhibited little cloudy sediment development compared with other samples. Such differences in the responses of oil samples to storage at 6°C may reflect differences in the supersaturation level, nature of the dissolved high-melting constituents and the presence of other oil components or contaminants that could affect the crystallization process (13). All three samples showed little sediment formation as measured by turbidity, during storage at 12°C (Fig. 1). Turkulov *et al.* (10) observed a higher rate of sediment formation when sunflower oil was stored at 13°C compared with storage at 0 and 5°C. The increased rate of sediment formation at the higher temperature was attributed to decreased viscosity which enabled more rapid diffusion of wax molecules, thereby promoting crystallization. For canola oil, however, the observed decreased turbidity at higher temperatures may be explained as the temperature dependence of nucleation and crystal growth. While both processes are favored at lower temperatures, nucleation (a prerequisite for crystallization) has a much lower optimum temperature compared with crystal growth (14). This is of practical significance with respect to the kinetics of the overall sedimentation process. Commercial oils are often exposed to fluctuating and low temperatures during transporation and warehousing compared with the temperature regimens used in this study. Such conditions would accelerate formation of sediment in the oil.

Effect of sediment removal on oil stability. After removing the sediment, the clarified canola oil samples were subjected to the same storage temperature regimes as previously described to test their stabilities. Turbidity formation was again found to occur faster at the lowest temperature (2°C) and to decrease as storage temperature increased (Fig. 2). Much smaller differences were observed between oil samples, with and without removal of sediment, when stored at 12°C. This was attributed to higher solubility and less supersaturation of the crystallizing constituents at this temperature (13). Decreased supersaturation combined with an elevated storage temperature would slow down the crystallization events (14). From these experiments, it is clear that removing the sediment from the oil prior to storage, while substantially reducing sedimentation, did not eliminate this phenomenon from the oil. In addition to the concentration of high-melting crystallizing compounds, it is conceivable that the presence of microparticulate foreign matter in the filter aid or other endogenous oil constituents could influence the metastability of the oil-sediment system (13). Formation of crystalline metastable structures of waxes and saturated triglycerides with phospholipids and soaps can prevent sediment formation and removal of these constituents from the oil. These complexes, with unpredictable stability, may release components that cause turbidity when their concentration has been changed (13,15-18).

Physicochemical characterization of sediments. The DSC thermal profiles for sediments with different levels of purity and origin are shown in Figures 3 and 4. Sediments obtained directly after filtration, when residual oil was present, gave wider melting transitions, even after washing once with petroleum ether (Fig. 3). Similar melting behavior has been reported for solid fats heated in liquid triglycerides. In the presence of an increased

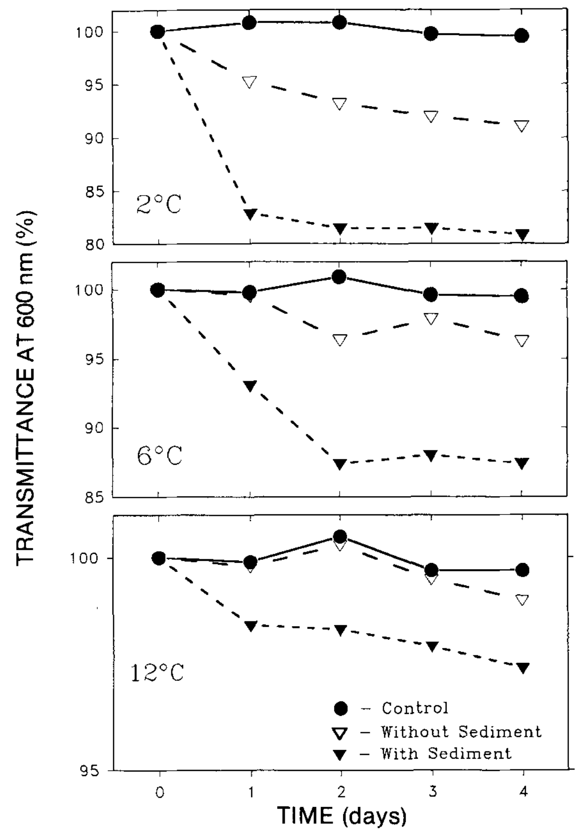

FIG. 2. Sediment formation in canola oil before and after sediment removal.

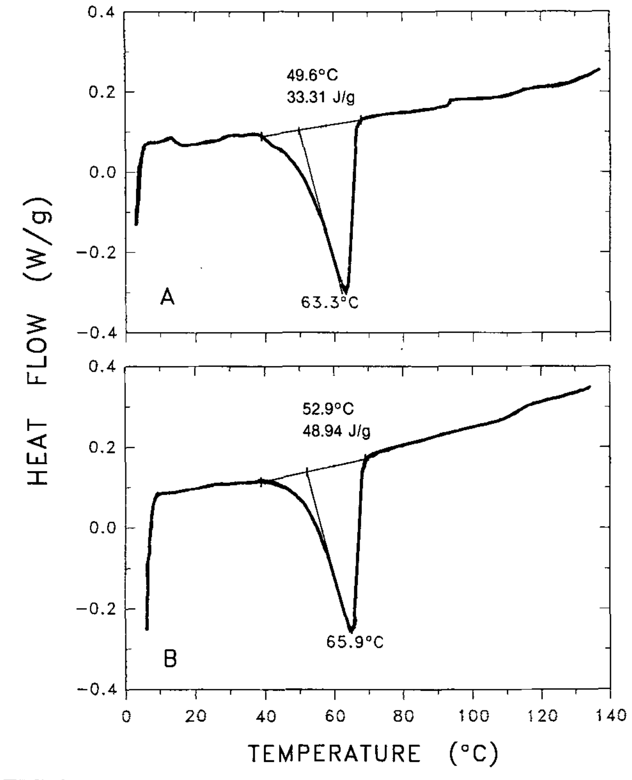

FIG. 3. Thermal curves of canola oil sediment (A) and commercial winterization sediment (B) after single washing with cold petroleum ether.

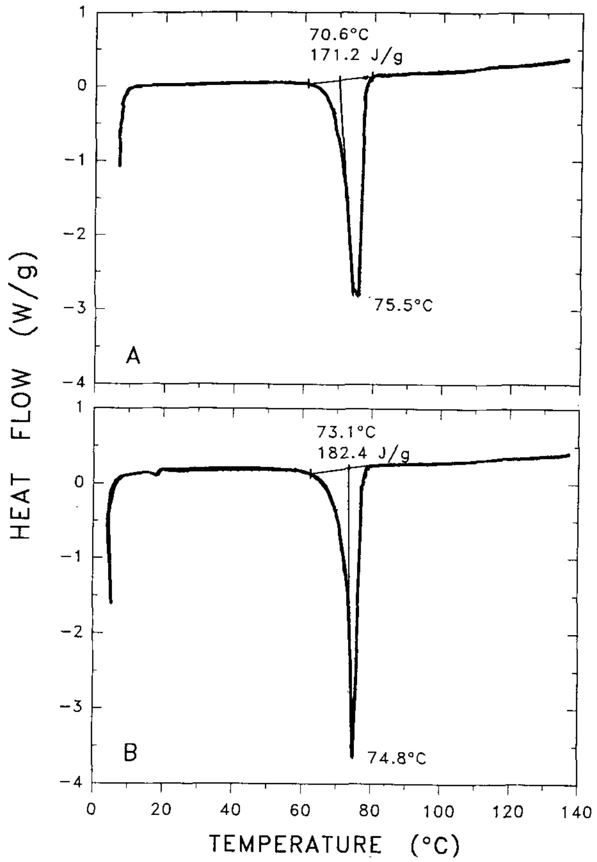

FIG. 4. Thermal curves of canola oil sediment (A) and commercial winterization sediment (B) after double washing with cold petroleum ether.

amount of liquid phase, the solid fraction dissolved in it also increases at any given temperature, thereby resulting in broader endothermic transitions at lower temperatures (19). Following the second washing with petroleum ether, the melting endotherms became narrower and appeared at higher temperatures (Fig. 4). The peak melting temperature of sediments was at 63, 66, 75 and 76°C for the single- and double-washed COS and IWS, respectively.

The sediment formed during methylation also gave sharp and narrow melting profiles with melting temperatures similar to the double-washed sediment from commercial winterization (Fig. 5). Chemical characterization of this sediment revealed that the main compounds were long-chain saturated alcohols similar in composition to the alcohols identified in IWS (Table 1).

Analysis of the sediments by GC–MS confirmed the presence of long-chain fatty acids and alcohols (Figs. 6 and 7). Individual compounds were identified and quantitated; the results are summarized in Table 1. Fatty acids and alcohols with 26 to 32 carbons atoms in the chain were found in canola oil. These long-chain compounds have not been reported in canola or rapeseed oils or seeds (20–24). Alcohols and fatty acids with chainlengths over 26 carbon atoms have been identified in many epicuticular waxes of higher plants. Many of these compounds have more than 30 carbon atoms in their chains (24).

Differences in fatty acid and alcohol compositions were found between the sediments (COS, IWS). Half of all the

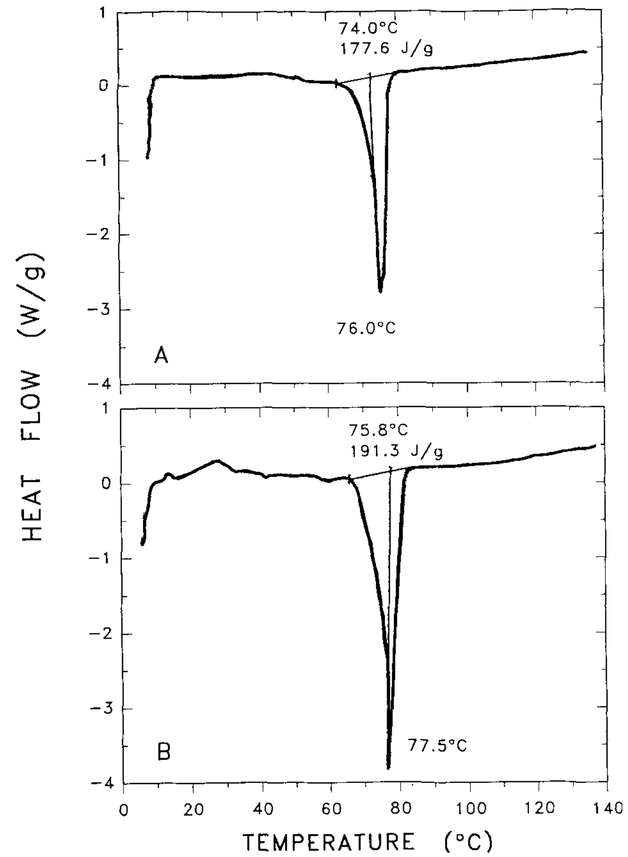

FIG. 5. Thermal curves of sediments formed after saponification of canola oil sediment (A) and commercial winterization sediment (B). See Methods for details.

fatty acids in COS was arachidic acid, while this acid contributed 35% of the total fatty acids in IWS. The latter sediment contained much more of the very long-chain fatty acids compared with COS (Table 1). The contribution of cerotic (C26:0), montanic (28:0), melissic (C30:0) and lacceroic (C32:0) acids were 6, 15, 27 and 14 times greater in the sediment from commercial winterization (IWS) than in the oil sediment (COS). IWS contained mainly saturated fatty acids with even numbers of carbon atoms in the chains.

The contribution of long-chain alcohols in both sediments is shown in Table 1. Alcohols with even carbon numbers and those with more than 26 carbon atoms in the chain were 6.2 times higher in IWS than in COS. IWS contained alcohols with 19 and 21 carbon atoms, which were not found in COS. Alcohols with 30 and 32 carbon atoms were 1.5 and 1.9 higher in IWS than in COS. In addition, contribution of tetracosanol (C24:0) was twofold higher in COS than in IWS.

Long-chain fatty acids comprised most of the esters of long-chain alcohols, although this does not exclude the possibility that the former could be a part of triglycerides. The direct result of this would be to increase their melting temperature and change the solubility, which can initiate crystallization even at room temperature. Waxes formed from very-long-chain fatty acids and alcohols will have high melting properties (13,25). Commercial winterization is generally carried out at a temperature range of 6–10°C to remove these high-melting compounds from the oil. This explains why sedimentation is caused by these long-chain fatty acids and alcohols, incorporated into the structure of high-melting compounds and transferred into the oil during processing of the seed.

TABLE 1

Composition of Sediments Isolated from Bottled Canola Oil and Filter Cake from Commercial Winterization[a]

	Contribution in sediment (%) from:			
	Canola oil (COS)		Winterization (IWS)	
Compound	Fatty acid	Alcohol	Fatty acid	Alcohol
C15:0	0.31	n.d.[b]	n.d.	n.d.
C16:0	2.38	0.18	1.83	0.56
C16:0B[c]	0.73	n.d.	1.27	n.d.
C17:0	0.24	n.d.	0.23	0.35
C18:0	3.36	0.43	1.81	0.43
C18:1	2.81	n.d.	n.d.	n.d.
C18:2	0.44	n.d.	n.d.	n.d.
C19:0	0.27	n.d.	n.d.	3.96
C20:0	54.45	2.06	35.46	0.46
C21:0	1.21	n.d.	0.83	6.73
C22:0	21.52	11.45	20.47	1.45
C23:0	0.57	0.93	0.72	4.97
C24:0	3.84	31.01	8.03	14.32
C25:0	0.23	2.45	0.65	5.03
C26:0	1.25	26.51	7.17	23.64
C27:0	n.d.	0.73	0.51	5.74
C28:0	0.63	10.47	8.97	12.22
C29:0	n.d.	0.41	0.43	1.76
C30:0	0.12	4.43	3.25	6.75
C31:0	n.d.	0.16	0.13	0.23
C32:0	0.03	3.23	0.42	6.27
Others[d]	5.61	5.55	7.82	5.13

[a] Abbreviations: COS, commercial oil sediment, IWS, industrial winterization sediment.
[b] Not detected.
[c] Fatty acids with branched chain.
[d] Compounds not identified.

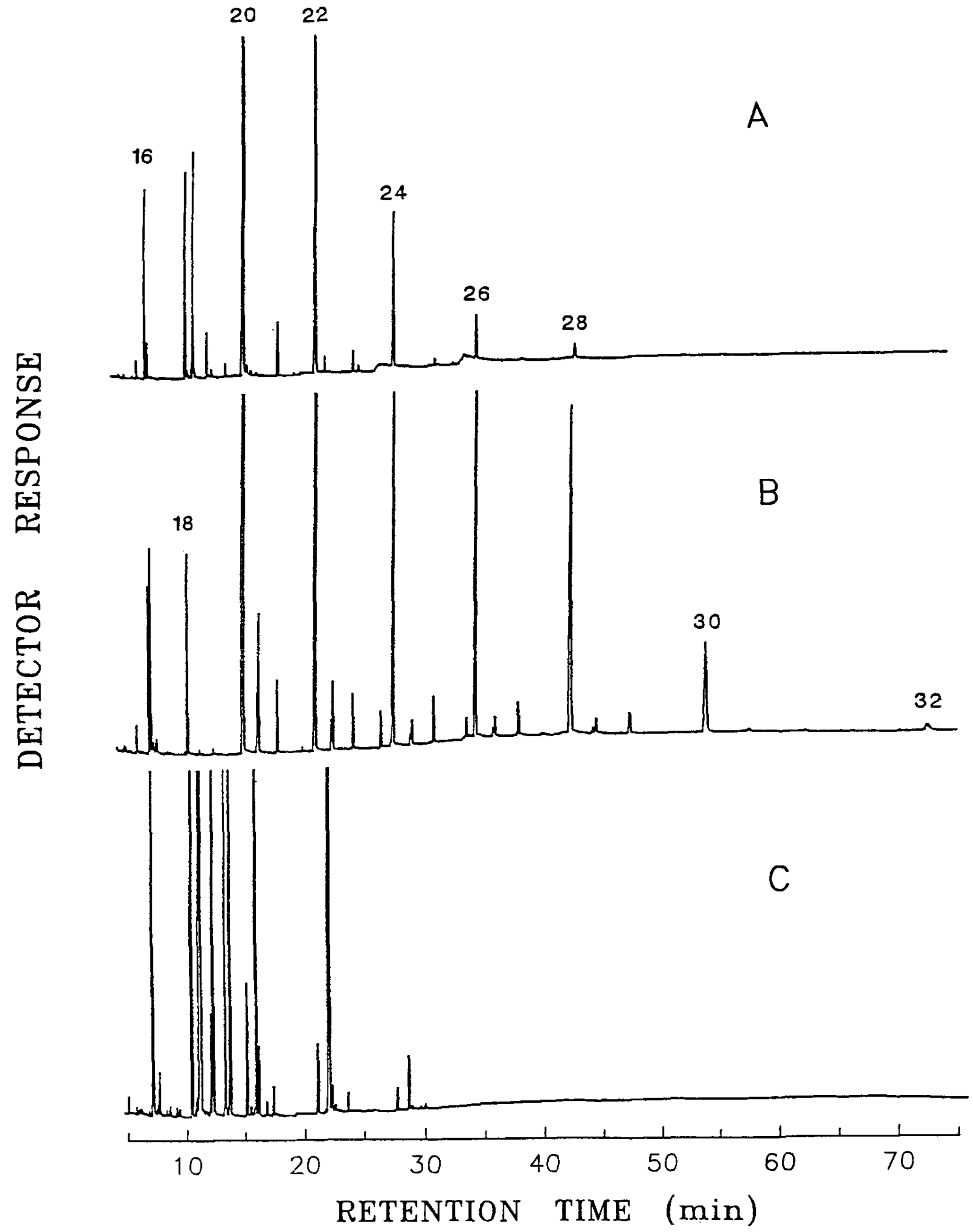

FIG. 6. Chromatograms of fatty acids isolated from canola oil sediment (A), commercial winterization sediment (B) and rapeseed oil (C). Numbers refer to carbon atoms of aliphatic chain.

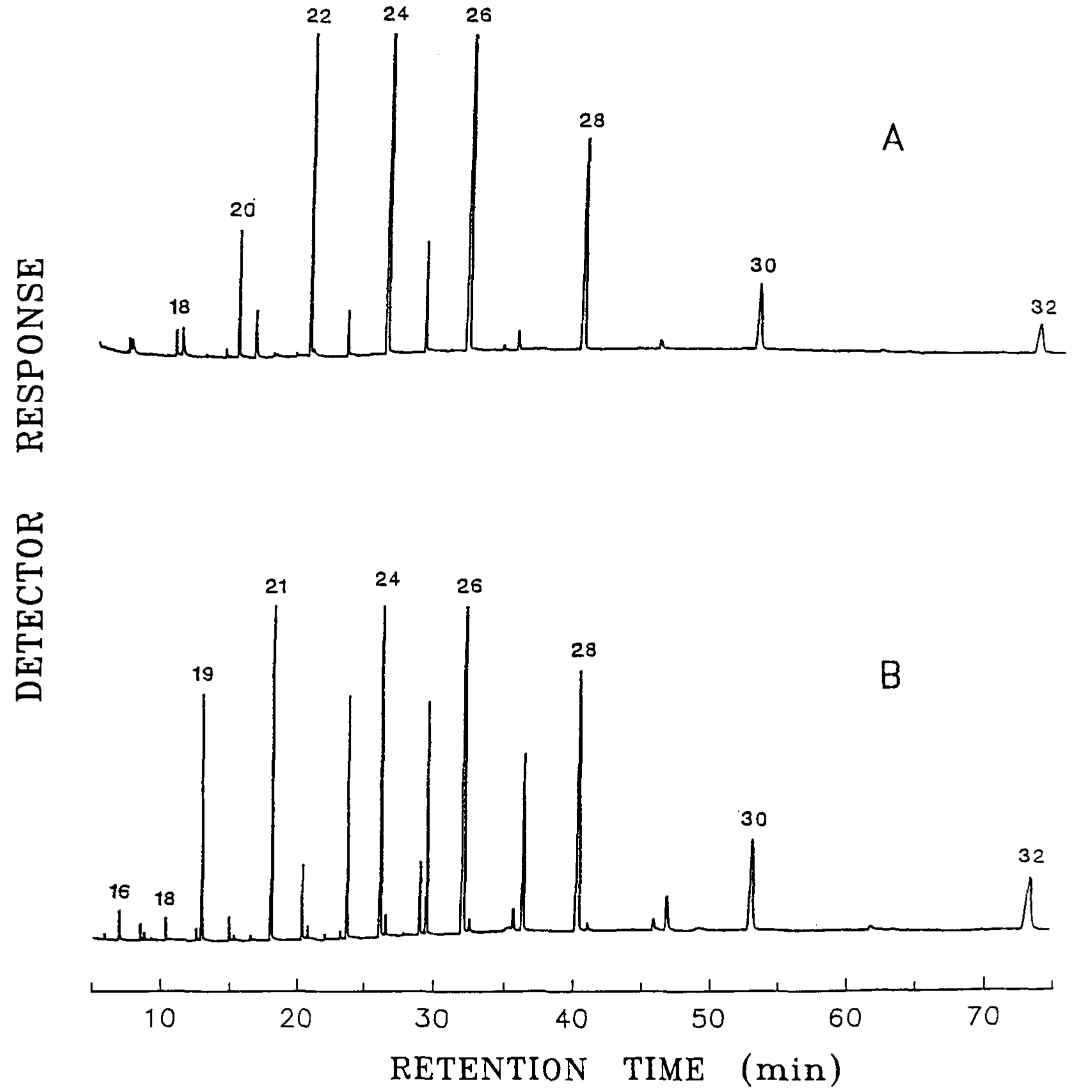

FIG. 7. Chromatograms of alcohols isolated from canola oil (A) and commercial winterization (B) sediments. Numbers refer to carbon atoms of the aliphatic chain.

These studies demonstrated, for the first time, the presence of long-chain compounds in canola oil that may cause sediment formation. The etiology of these compounds remains to be established. Further studies are underway to predict the occurrence of this phenomenon and to eliminate it altogether.

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