Evaluation of the Role of Saturated Fatty Acids in Sedimenting Canola Oils

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ABSTRACT: Canola oil is generally a clear oil which does not require winterization. However, sediment formation has become an increasing problem in Australian canola oil. Canola oil stored at temperatures between –5 and 21°C formed sediment more rapidly at lower temperatures. The sediment and clear fractions of a group of sedimenting canola oils were analyzed and compared. Both fractions contained wax esters of carbon number C42–C52, the sediment fractions containing between 0.37 and 3.09 mg g^{-1} and clear fractions containing between 0.12 and 0.85 mg g^{-1}. The triacylglycerol profiles of sediment fractions contained four compounds, PPO, PPP, PSO and PPS (where P is palmitoyl, O is oleoyl, and S stearoyl), that were not detected in clear fractions. The contents of palmitic acid and total saturated fatty acids were higher in the sediment fraction than the clear fraction. Added PPP clouded a clear oil as effectively as stearyl behenate and more than OOO or lauryl arachidate. Sedimentation may be linked to environmental conditions, as seed grown in 1997, a dry year, produced more problem oils than seed grown in previous years that had more nearly average rainfall.

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KEY WORDS: Canola oil, saturated fatty acids, sediment, shelf test, tripalmitin, wax esters.

Canola oil is considered a premium oil owing to its favorable fatty acid profile and tendency to remain clear at reduced temperatures. The latter property is an economic advantage as canola oil has not required winterization, unlike other vegetable oils such as sunflower oil. However, concerns about cloud and sediment formation in refined Australian canola oils have been expressed in recent years. The reasons for this apparent change may lie in the rapid increase in canola oil usage in Australia and marketing in clear bottles. However, the extent of the problem has been variable over the last four growing seasons, suggesting that the problem is real rather than perceived. In 1995 and 1996, cloudiness in Australian canola oil did not cause a significant problem for the industry. Considerable quantities of canola oil produced from seed grown in 1997 have developed sediment after refining and bottling (Bowrey, R., Meadow Lea Foods, personal communication).

Several canola-growing areas suffered unusually dry conditions during 1997 which have a significant effect on canola grain quality (1,2). Of most significance in quality is the variation in oil content from around 35 to as much as 50% oil. Oil and protein contents generally show an inverse relationship, and a hot, dry finish to the canola growing period results in low oil and high protein concentrations. Fatty acid profiles are also influenced directly by growing temperature (3) with high temperatures favoring reduced polyunsaturated fatty acid content.

The sediment or cloud is often referred to as "wax" (4–6). Although its precise composition remains controversial, it is now accepted as a mixture of components including triacylglycerols (TAG), free fatty alcohols, wax esters, and hydrocarbons (7). For example, Liu *et al.* (5,8,9) reported 72–80% of the canola sediment consisted of wax esters whereas Gao and Ackman (6) indicated that the sediment contained in excess of 90% TAG.

The commercial significance of this problem has ensured continuing interest in the causes of sediment formation. The role of storage temperature has been studied in vegetable oils including canola and sunflower oils. The critical temperatures for rapid sedimentation have been reported as 5°C (10) and 2°C (11) in canola oil and 5°C for sunflower oil (12). Crystal growth and structure in the sediment have been studied in depth (5,9,13,14). In palm olein, Sulaiman *et al.* (15) identified TAG, in particular, PPP, PPM and PPS where P = palmitoyl, M = myristoyl, S = stearoyl), as being critical to the formation of nuclei and thus clouding in refined bleached deodorized (RBD) palm olein.

The present study has compared the concentrations of the major components of the clear and cloudy fractions of a collection of refined canola oils. These have then been related to environmental conditions for 1996, a year in which there were virtually no sediment problems, and 1997, in which canola seed produced considerable problems for the industry. We found that the sediment fractions contained more wax esters, palmitic acid, and total saturated fatty acids than the corresponding clear fractions. Four palmitic acid-containing TAG were detected in the sediment fraction alone. The differences in composition of the two fractions suggests that these compounds are important in the sedimentation of canola oil.

MATERIALS AND METHODS

All solvents were chromatographic grade and were purchased from Selby-Biolab (Mulgrave North, Australia). TAG stan-

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dards and fatty acid methyl ester reference standards (Oil reference standard, AOCS, O-7756) obtained from Sigma-Aldrich (Castle Hill, Australia) included the olive oil TAG reference kit (TRI-5), PPP (T5888), PPO (D2282), PPL (D0176), and PLL (D0301), where L is linoleoyl. Wax ester standards, palmityl palmitate, lauryl arachidate, stearyl stearate, behenyl heptadecanoate, arachidyl arachidate, stearyl behenate, and behenyl behenate, were purchased from Nu-Chek-Prep (Elysian, MN).

Commercial oil samples were obtained from local supermarkets and manufacturers and were characterized on the conditions of sediment formation at low temperatures (canola oils, samples C11–C14); room temperature (canola oils, samples C1–C10; sunflower oils, samples S1–S3; vegetable oils, samples V1 and V2; peanut oil, sample P1); or no sedimentation at all (canola oil, sample C15). A filter cake sample from canola oil, F1, was also considered.

Shelf test. Canola oil sample C11 was selected for use in the shelf test because of its behavior in sediment formation at room temperature. Subsamples of the oil were stored in individual bottles for a week at room temperature to ensure complete dissolution of any sediment. Bottles were then stored at various temperatures between –5 and 22°C and observed at regular intervals for sediment formation, which was scored on a linear scale from 0 (no visible cloud) to 7 (large flakes, settling to the bottom of the bottle).

Isolation of sediment and clear fractions. Sediment was isolated from oil samples by centrifugation for 20 min in a Sorvall refrigerated centrifuge (Du Pont Australia, North Ryde, Australia) at 5000 rpm and 2°C using precooled containers. The supernatant was removed and the sediment recentrifuged until no further supernatant was apparent. The clear fraction was obtained from the top of sample bottles and centrifuged once in the same manner to remove any sediment traces.

Melting point. The melting points of sediments isolated from shelf test samples were determined according to AOCS method Cc 1-25 (16).

Thin-layer chromatography. The sample (1.0 g) was mixed with chloroform (100 μL) and internal standard (100 μL, 1 mg mL^{-1} lauryl arachidate in chloroform) and applied to silica gel 60 TLC plates (Merck, Kilsyth, Australia) that had been activated by heating at 100°C for 1 h and then cooled in a desiccator. Plates were loaded with 140 μL of sample and developed with hexane/diethyl ether/acetic acid (80:20:1 vol/vol/vol) (17). Standards (5 μg lauryl arachidate and 5 μg PPP) were applied in the first lane of the plate. Plates were sprayed with 2,7-dichlorofluorescein (0.2% in ethanol) to visualize the separated components.

Wax esters. The wax ester band was scraped from the TLC plate and soaked overnight in chloroform (10 mL), then washed twice with a further 5 mL chloroform. Solvent was removed with a rotary evaporator at 40°C and then under nitrogen to a final volume of 0.50 mL.

Extracts were analyzed using a Varian model 3400 gas chromatograph (Mulgrave, Australia) and a BPX-5 fused-silica column (10 m × 0.25 mm i.d.; 0.25 μm film) (SGE, Ringwood, Australia). Column temperature was programmed from 80 to 120°C at 30°C min^{-1}, then to 345°C at 5°C min^{-1}, and then held at 345°C for 10 min. Helium was used as the carrier gas at 5 mL min^{-1} with on-column injection (320°C) and flame-ionization detection at 350°C. Eluted species were identified by comparison with retention times of wax ester standards, and integration was performed using Varian Star Chromatography Workstation software, version 4.5.

TAG. The sample (0.1 g sediment or clear oil) was dissolved in dichloromethane (5 mL) and analyzed on a Waters high-performance liquid chromatography (HPLC) system (Waters, Rydalmere, Australia) equipped with a WISP 712 autosampler, using a 15 μL injection. Separation was performed on a Wakosil C18 column (25 cm × 4.6 mm, 5 μm) (SGE) using isocratic elution with 32% dichloromethane in acetonitrile over 120 min. TAG were detected with a Sedex 55 Evaporative Light Scattering Detector (ELSD) (Sedere, Alfortville, France) and integrated using Millennium^{32} software, version 3.05. Identification of peaks was performed by comparison with standards and also by liquid chromatography–mass spectrometry (LC–MS).

LC–MS was performed on a Hewlett-Packard 1090 (Blackburn, Australia). Separation was performed isocratically as described above, with 150 μL min^{-1} directed to the electrospray source of the mass spectrometer. A Quattro II triple quadropole mass spectrometer (Micromass, Altrincham, Cheshire, United Kingdom) fitted with an electrospray source was used to record the electrospray ionization spectra. Alternating mass spectrometer scan functions were performed. Scan function 1 consisted of a 3-s, positive ion scan from *m/z* 500 to 1200 at a cone voltage of 30 V, while scan function 2 was a 3-s, positive ion scan from *m/z* 50 to 1000 at a cone voltage of 100 V.

Fatty acid profile. The fatty acid profile was determined on both sediments and clear oils following methylation. The sample (0.1 g sediment or clear oil) was mixed with petroleum ether (3 mL). Sodium methoxide (0.5 mL, 2.3 g sodium in 200 mL anhydrous methanol) was added and the mixture vortexed for 15 s. After standing for 10 min, bromothymol blue indicator (2–3 drops) was added and the mixture vortexed for 5 s. Hydrochloric acid (HCl) (0.1 mL, 1 N) was added followed by sodium carbonate (0.6 mL, 1.5% in water) and mixed. Distilled water was added to bring the petroleum ether layer to the top of the test tube, then the top layer was removed for analysis.

Samples were analyzed using a Varian model 3800 gas chromatograph with a BPX-70 fused-silica column (30 m × 0.25 mm i.d.; 0.25 μm film) (SGE). Column temperature was programmed at 180°C for 8 min, then to 220°C at 10°C min^{-1}, and finally held for 4 min. Helium was used as the carrier gas at 1 mL min^{-1} with a split/splitless injector (250°C) and a flame-ionization detector (260°C). Integration was performed using Varian Star Chromatography Workstation software, version 4.5.

Sediment tests. Sample C15 was a clear oil that showed no sediment formation under the conditions employed in this study. PPP, OOO, stearyl behenate, lauryl arachidate, or a mixture of PPP and lauryl arachidate were each added to the oil in
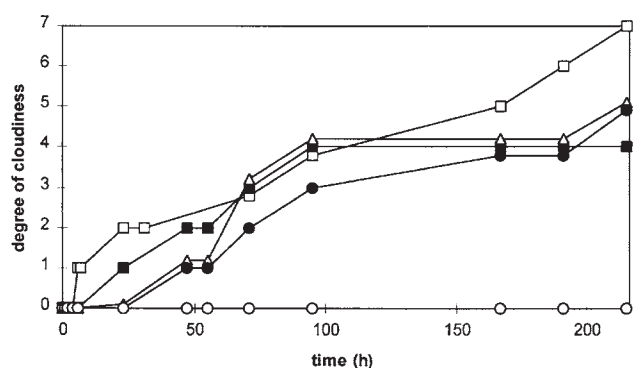
FIG. 1. Effect of temperature in sediment formation. Subsamples of canola oil sample C11 were first held for 1 wk at room temperature to ensure dissolution of any sediment, then stored at (□) –5°C, (■) 4–7°C, (△) 5°C, (●) 9°C, and (○) 22°C and observed for sediment formation, which was scored on a linear scale from 0 (no visible cloud) to 7 (large flakes, settling out).

concentrations up to 3 mg mL^{-1}. The oils were then heated to 100°C for 5 min, shaken to ensure complete dissolution of the added material, cooled to room temperature, stored at 9°C and observed at regular intervals to 7 d.

Moisture availability. Moisture availability was modeled for 1996 and 1997 using a computer-based model (18). This model uses daily rainfall and pan evaporation data to calculate moisture available to canola crops growing at Wagga Wagga.

RESULTS AND DISCUSSION

Shelf test data (Fig. 1) established that sediment formation in canola oil occurred more rapidly at lower temperatures. For instance, oil stored at –5°C began to cloud within 6 h whereas the oil stored at 9°C did not cloud until 25 h. Oil stored in a storeroom refrigerator that varied in temperature between 4 and 9°C began to cloud sooner than oil stored at either 5°C or 9°C, but sediment formation had slowed by the end of the observation period. In a study on sunflower oil, Chulu *et al.* (12)

found that oil stored at 25°C for 21 h and 5°C for 3 h clouded faster than oil kept consistently at 25°C, but slower than oil kept at 5°C.

Oil stored at 22°C, which approximates supermarket storage conditions, showed no sediment or cloud formation after 4 mon of storage even though the sample had originally formed and retained a sediment in the supermarket. This indicates the complexity of the problem and the difficulty of devising suitable storage tests for prediction of sediment behavior.

The melting point of the canola sediment formed at both 5 and 9°C was 37.8°C. This is considerably lower than the value of 74.8–77°C reported by Liu *et al.* (9,10) for canola filter cake sediments which had "oil residue" removed by two washes with cold solvent. Similarly, Przybylski *et al.* (11) reported a melting point for canola industrial winterization sediment of 66°C after one wash with cold solvent and 75°C after a second wash. However, Przybylski also found a lower melting point of 63°C for commercial canola oil sediment after one wash and 76°C after a second wash. This suggests that washing with cold solvents selects for higher melting point components, which then were used in the respective studies.

From the TLC data, the major components of the canola sediment were TAG with smaller amounts of wax esters, hydrocarbons, and more polar compounds, most probably free fatty acids and free fatty alcohols. This is in sharp contrast with other work in which wax esters have been reported (4,5,10,11,14) as the major constituent of canola sediment. This difference can be attributed to the method of sample preparation. Previous studies used sediment samples from which the majority of the TAG content had been removed from the sediment prior to analysis by washing with cold solvents (5,10,11,14). Wax esters identified in both sediment and clear oil ranged in carbon number from C42 to C52 (Table 1). Chromatograms contained three peaks which overlapped the region of C48 to C52, which has been excluded from the calculations. Gao and Ackman (6) identified these peaks as three sterol esters found in canola oil, for the ratio of brassicasterol to campesterol to sitosterol (5:35:60, respectively) is similar to

TABLE 1
Wax Esters (mg g^{-1}) in Clear and Sediment Fractions[a]

	Wax esters, carbon number							
	C42	C43	C44	C45	C46	C47	C52	Sum
C2 clear fraction	0.00	0.01	0.00	0.01	0.02	0.12	0.00	0.17
C2 sediment fraction	0.15	0.05	0.35	0.17	0.21	0.11	0.00	1.05
C3 clear fraction	0.03	0.05	0.06	0.09	0.22	0.41	0.00	0.85
C3 sediment fraction	0.10	0.01	0.48	0.23	0.66	0.54	0.01	2.03
C6 clear fraction	0.01	0.00	0.00	0.00	0.01	0.10	0.00	0.12
C6 sediment fraction	0.16	0.01	0.13	0.00	0.05	0.12	0.00	0.48
C7 clear fraction	0.06	0.03	0.04	0.05	0.11	0.20	0.01	0.51
C7 sediment fraction	0.07	0.02	0.23	0.09	0.15	0.07	0.02	0.65
C8 clear fraction	0.02	0.05	0.02	0.01	0.03	0.04	0.00	0.17
C8 sediment fraction	0.32	0.47	0.60	0.24	0.87	0.59	0.00	3.09
C14 clear fraction	0.03	0.04	0.03	0.02	0.02	0.09	0.00	0.22
C14 sediment fraction	0.05	0.05	0.11	0.03	0.04	0.08	0.01	0.37
F1	0.07	0.09	0.39	0.30	0.54	0.64	0.01	2.04

[a]Sediments formed at room temperature: canola oil samples C2, C3, C6, C7, C8; sediments formed only when refrigerated: canola oil sample C14; sample of canola filter cake, F1.
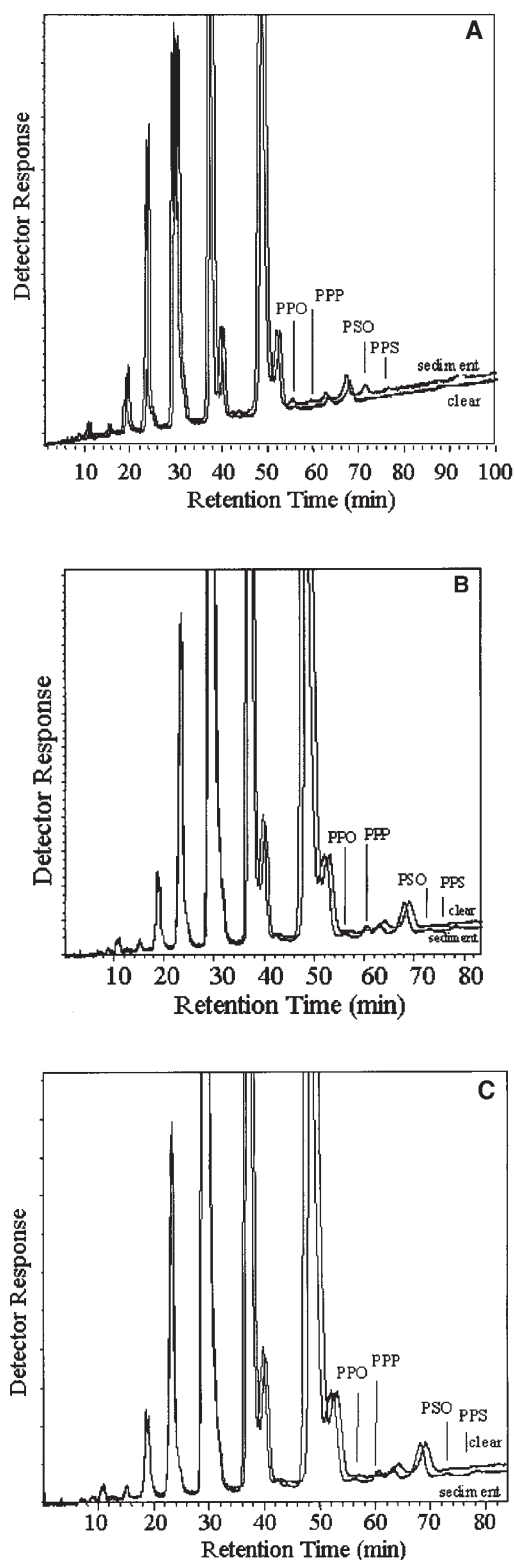
FIG. 2. Chromatograms of the sediment and clear fractions of three canola oils: samples C1 and C6, both of which formed sediments at room temperature, and C14, which formed a sediment only when it was refrigerated. Chromatograms of sediments contain four peaks that elute between 55 and 85 min and that are not present in clear oil. Abbreviations: PPP, tripalmitin; PSO, palmitoyl-stearoyl-oleoyl glycerol; PPS, dipalmitoyl-stearoyl glycerol; PPO, dipalmitoyl-oleoyl glycerol.

the ratio of these three peaks. Comparison with standards in this study shows that sterol esters elute much earlier, while TAG elute in the region in question. The sediment fractions contained between 0.37 and 3.09 mg g^{-1}, and the clear fractions contained lower quantities in each sample, between 0.12 and 0.85 mg g^{-1}.

TAG were determined in clear and sediment fractions of canola oils by HPLC–ELSD (Table 2). The proportions of the major TAG were similar in both fractions. However, sediment fractions consistently contained four additional compounds not detected in the corresponding clear fractions (Fig. 2). These compounds were identified by LC-MS as the TAG PPO, PPP, PSO, and PPS (Fig. 3).

TAG have been implicated in the sedimentation of palm oil (15). Samples taken from progressive stages of sediment formation showed an increased amount of PPP, MPP, and PPS compared to the starting oil, particularly in the early stages. These TAG are thought to contribute to nucleation, and the four TAG observed in this study may act in a similar fashion. From a consideration of melting behavior, PPP (melting point 66°C) is more likely to be implicated in sediment formation than either OOO (–4 to –5°C) or SSS (55°C).

TAG data are supported by fatty acid profiles (Table 3) of sediments and clear oils. The total saturated fatty acids were higher in all sediment fractions than the corresponding oils (Table 4), as also reported by Hu *et al.* (4) for the isolated TAG fraction of canola sediment. The present data show that palmitic acid in particular was elevated in the sediments, consistent with the appearance of the four additional TAG. On the other hand, C18:1 and C18:3 were generally lower in canola sediments (Table 3). Saturated fatty acids are high-melting point compounds which may act in either nucleation or growth of crystals.

Differences were observed in the ability of PPP and OOO to cloud an otherwise clear oil (Table 5). PPP was effective in producing heavy sediment to 0.3 mg mL^{-1}, and lighter sediment at 0.1 and 0.05 mg mL^{-1}. OOO did not produce sediment at any concentration tested. The ability of PPP to precipitate and otherwise clear oil suggests the possible role of saturated TAG in canola oil sedimentation. Sediment from a sample containing PPP added at 6.25 mg mL^{-1} was analyzed by HPLC and showed a representative chromatogram with a significant amount of PPP. Sulaiman (15) found higher levels of PPP, MPP, and PPS in palm oil sediment. These TAG were particularly pronounced in the early stages of crystal formation, suggesting an important role in crystal nucleation. The four TAG identified in this study could have a similar role in the nucleation of crystals in canola oil, which is followed by the active or inactive involvement of other TAG.

Long-chain wax esters have been identified as a major component in canola oil sediment samples that have been washed free of TAG (4,5,10,11,14). Stearyl behenate (C40) produced clouding similar to PPP. Lauryl arachidate (C32) produced some clouding at higher concentrations, but the oils remained clear below 0.5 mg mL^{-1}. This experiment shows that the longer-chain wax esters are more effective at causing sedimentation than the shorter-chain wax esters. Oil to which both PPP
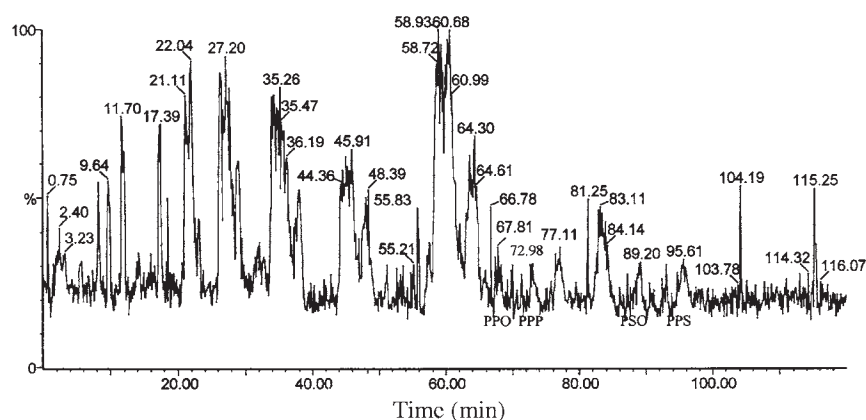
FIG. 3. Liquid chromatography–mass spectrometry chromatogram of a sedimenting oil (sample C8). Peaks of interest are PPO (dipalmitoyl-oleoyl glycerol, 67.81 min), PPP (72.98 min), PSO (89.20 min), and PPS (95.61). For abbreviations see Figure 2.

TABLE 2
Triacylglycerol Profiles for the Clear (clr) and Sediment (sed) Fractions of Four Problem Oils[a]

TAG	C1 clr	C1 sed	C6 clr	C6 sed	C12 clr	C12 sed	C14 clr	C14 sed
LLnLn	0.15	0.07	0.18	0.14	0.09	0.10	0.06	0.10
LLLn + OLnLn + PLnLn	1.96	1.93	1.72	1.76	1.24	1.16	1.63	1.33
LLL + OLLn + PLLn	7.32	7.12	7.08	7.04	5.94	5.49	7.21	6.18
PSS	4.88	5.41	6.77	7.98	7.13	6.59	5.81	5.08
OLL + OOLn + PLL + POLn	17.47	16.71	14.80	13.07	12.36	12.33	14.80	15.50
OOL + SLL + SOLn	24.95	24.46	26.16	25.44	24.87	26.06	26.28	25.64
POL	3.19	3.30	3.31	3.77	4.78	3.41	3.32	4.06
OOO	35.09	34.60	35.30	34.00	36.26	37.34	35.76	36.89
POO + PSL	3.80	3.93	3.55	4.33	5.82	4.32	3.93	3.16
PPO	0.00	0.18	0.00	0.19	0.00	0.09	0.00	0.30
PPP	0.00	0.14	0.00	0.21	0.00	0.01	0.00	0.06
Unknown	0.26	0.30	0.21	0.25	0.25	0.34	0.27	0.20
SOO	0.92	1.01	0.91	1.35	1.25	1.44	0.92	0.84
PSO	0.00	0.29	0.00	0.17	0.00	0.54	0.00	0.32
PPS	0.00	0.12	0.00	0.23	0.00	0.79	0.00	0.34
Unknown	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00
Unknown	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00

[a]Canola oil samples C1 and C6 formed sediments at room temperature; canola oils C12 and C14 formed sediments only when refrigerated. Abbreviations: TAG, triacylglycerol; L, linoleoyl; Ln, linolenoyl; O, oleoyl; P, palmitoyl; S, stearoyl.

and lauryl arachidate had been added sedimented more heavily than oil containing added lauryl arachidate alone, but slightly less than oil with an equivalent amount of PPP.

It is significant that PPP and stearyl behenate are similar in their ability to cloud a clear oil. Although wax esters have been identified as a major component in canola sediment, sediment samples are often washed free of TAG because they are not considered to play a part in sedimentation. This study shows that saturated TAG have an important role in canola oil sedimentation and are able to cause sedimentation in otherwise clear oil.

Australian canola suffered widespread cloud and sedimenting problems in oil processed from seed grown in 1997. Seed grown in 1996 did not result in sedimenting oils. This may be related to the environment, as conditions in Australia were much drier in 1997 than 1996. Figure 4 illustrates the levels of moisture available to canola grown at Wagga Wagga in 1996 and 1997. A value above 0.5 indicates sufficient available moisture, while a value below 0.5 indicates that the plant is water stressed. The occurrence of the sediment problem in the drier year and not in the better year suggests a link between environmental conditions and the sediment problem, with drier years producing seed containing high levels of saturated fatty acids, particularly as saturated TAG, and high levels of long-chain wax esters.

TABLE 3
Fatty Acid Profiles of Clear and Sediment Fractions of a Range of Commercial Oils[a]

		14:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0	24:1
C1	Clear fraction	0.09	4.31	0.20	0.11	0.07	2.03	62.93	17.82	9.22	0.61	1.33	0.34	0.45	0.23	0.26
	Sediment fraction	0.26	6.12	0.19	0.20	0.06	4.73	59.36	16.98	8.68	0.78	1.28	0.40	0.42	0.31	0.21
C2	Clear fraction	0.08	4.77	0.25	0.11	0.06	2.21	61.86	19.82	8.00	0.61	1.22	0.32	0.38	0.18	0.13
	Sediment fraction	0.1	5.46	0.16	0.14	0.05	3.41	59.53	20.74	7.82	0.57	1.09	0.32	0.31	0.16	0.12
C3	Clear fraction	0.07	4.63	0.23	0.11	0.06	2.23	62.07	19.76	7.84	0.62	1.22	0.37	0.39	0.23	0.17
	Sediment fraction	0.56	6.27	0.28	0.24	0.34	4.81	55.91	20.9	7.86	0.65	0.97	0.38	0.4	0.24	0.19
C4	Clear fraction	0.05	3.85	0.19	0.10	0.05	2.09	62.71	18.99	9.13	0.63	1.24	0.32	0.30	0.20	0.16
	Sediment fraction	0.07	4.92	0.18	0.14	0.05	1.83	61.78	21.10	7.62	0.52	0.96	0.27	0.24	0.18	0.14
C5	Clear fraction	0.05	4.16	0.23	0.10	0.06	2.06	61.86	19.89	8.90	0.57	1.17	0.29	0.29	0.22	0.15
	Sediment fraction	0.07	5.32	0.24	0.15	0.06	1.95	59.93	21.80	7.97	0.51	1.03	0.26	0.25	0.31	0.15
C12	Clear fraction	0.05	3.74	0.16	0.09	0.05	2.10	61.99	21.97	6.66	1.14	1.18	0.30	0.22	0.21	0.13
	Sediment fraction	0.06	4.48	0.19	0.11	0.06	2.93	64.11	18.68	6.31	0.82	1.16	0.41	0.24	0.28	0.16
C15	Whole oil	0.06	4.79	0.17	0.09	0.05	2.05	66.04	23.54	0.43	0.59	1.22	0.33	0.25	0.20	0.19
S1	Clear fraction	0.09	6.30	0.08	0.05	0.04	4.58	25.89	61.16	0.13	0.32	0.19	0.75	0.04	0.28	0.10
	Sediment fraction	0.11	6.75	0.08	0.06	0.03	5.01	25.83	60.09	0.13	0.32	0.19	0.84	0.09	0.35	0.11
S2	Clear fraction	0.06	6.09	0.05	0.05	0.03	4.46	24.18	63.20	0.39	0.31	0.13	0.75	0.07	0.21	0.03
	Sediment fraction	0.10	6.44	0.25	0.10	0.05	4.56	23.70	62.03	0.80	0.71	0.24	0.71	0.04	0.25	0.02
V1	Clear fraction	0.07	9.65	0.06	0.10	0.05	3.49	21.85	56.03	7.76	0.28	0.16	0.31	0.02	0.15	0.02
	Sediment fraction	0.08	12.42	0.05	0.13	0.05	6.76	20.51	51.37	7.22	0.60	0.14	0.51	0.00	0.15	0.01
V2	Clear fraction	0.07	9.73	0.05	0.07	0.04	3.92	27.11	51.75	5.94	0.40	0.23	0.49	0.02	0.16	0.02
	Sediment fraction	0.09	10.11	0.05	0.08	0.04	4.52	26.86	50.95	5.74	0.47	0.22	0.52	0.04	0.27	0.05
P1	Clear fraction	0.03	8.80	0.06	0.07	0.04	2.09	45.65	36.30	0.00	1.10	1.26	2.91	0.12	1.54	0.00
	Sediment fraction	0.05	10.00	0.04	0.09	0.05	2.92	47.38	31.23	0.19	1.22	1.36	3.55	0.11	1.80	0.01

[a]Samples that formed sediments at room temperature: canola oil, C1–C5; sunflower oil, S1 and S2; vegetable oil, V1 and V2; peanut oil, P1. Canola oil that formed sediments only when refrigerated, C12, canola oil that remained clear even under refrigeration, C15. Data are expressed as normalized percentages.

TABLE 4
**Sum of the Saturated Fatty Acids in the Fatty Acid Profile of Clear Oil and Sediment[a]**

Sample	Clear fraction	Sediment fraction
C1	7.72	12.80
C2	8.28	10.16
C3	8.26	13.15
C4	7.24	7.93
C5	7.45	8.57
C12	7.96	8.99
S1	12.37	13.45
S2	11.93	12.87
V1	14.05	20.65
V2	14.84	16.06
P1	16.54	19.63

[a]For sample identification see Table 3. Data are expressed as normalized percentages.

TABLE 5
Sedimentation of an Otherwise Clear Oil[a]

	Amount of additive (mg mL^{-1})								
Additive	3.0	1.0	0.5	0.3	0.1	0.05	0.03	0.01	0
PPP	++++	++++	++++	++++	++	+	—	—	—
OOO	—	—	—	—	—	—	—		
Stearyl behenate	++++	++++	++++	++++	++	+	—	—	—
Lauryl arachidate	++	+	—	—	—	—	—	—	—
PPP and lauryl arachidate	++++	+++	++	++	—	—	—	—	—

[a]To sample C15, a clear canola oil that showed no sediment formation under the conditions employed in this study, was added 0–3 mg mL^{-1} additive. Oils were then heated to 100°C for 5 min, shaken, cooled to room temperature, stored at 9°C, and observed for 7 d. Symbols are: Clear, no sediment or cloud, + = tiny crystals, ++ = mid-sized crystals, +++ = large flakes, ++++ = large flakes, settling.
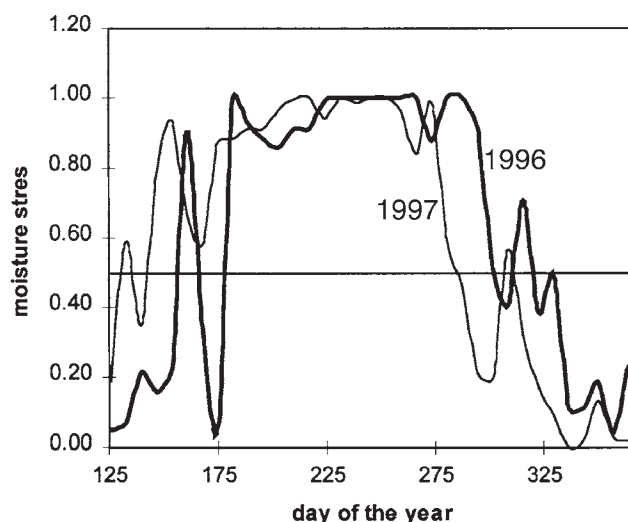
FIG. 4. Moisture availability at Wagga Wagga, Australia. Seed grown in 1997 finished the growing season with much less available moisture than seed grown in 1996 [calculated using a moisture availability model—software developed by G.M. Murray and P.S. Cornish (18)]. The line at 0.5 indicates sufficient available moisture.

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