Identification of Major Triglycerides Causing the Clouding of Palm Olein

P.Z. Swe[a], Y.B. Che Man[a,*], H.M. Ghazali[b] and L.S. Wei[c]
[a]Department of Food Technology and [b]Department of Biotechnology, Universiti Pertanian Malaysia, Malaysia, and [c]Department of Food Science, University of Illinois, Urbana, Illinois 61801

Clouding was obtained by storing palm olein at 12.5°C for up to 24 h and was separated by centrifugation. The fat crystals were collected after washing with cold acetone. The crystals were identified according to their fatty acid and triglyceride composition, carbon number and degree of unsaturation. Palmitic–oleic–palmitic (POP) and palmitic–oleic–stearic (POS) levels were high in the cloud material, especially in that recovered between 15–18 h of storage. The increase in POP and POS concentrations was concomitant with a decrease in the content of palmitic–oleic–oleic (POO). The least amount of POO was also obtained in clouds collected between 15–18 h of storage, compared to the original oil sample. The increase of palmitic acid explained that triglycerides conjugated with palmitic acid molecules in their acyl chains could have been crystallized, and crystallization could have taken place in the order of the number of palmitic molecule in the acyl chain. The concentrations of the other triglycerides did not change much throughout the storage period. Further storage caused the composition of the cloud to become similar to that of the original oil sample.

KEY WORDS: Carbon number, clouding, palm olein, POO, POP, POS, triglycerides.

Refined, bleached and deodorized (RBD) palm olein, commonly known as cooking oil, is mainly composed of 46% saturated fatty acid (myristic, palmitic and stearic), 43% monounsaturated fatty acid (oleic) and 11% polyunsaturated fatty acid (linoleic) (1). It is fully liquid at temperatures above 25°C but starts to crystallize, forming clouds, at lower temperatures. Cloud is the visible suspension of fat crystals normally seen in the container of palm olein stored at low temperature or after long storage at room temperature (about 25°C). Consumers often associate clouding with low-quality oil. Therefore, cloud formation must be prevented to improve oil quality. This requires identification of the triglycerides that make up clouds, so that a more efficient processing method can be developed that will result in cloudless palm olein.

The higher cloud point of palm olein, compared to any other liquid oils, has been a problem over the years and has been partially overcome by blending, adding additives or using a double fractionation method, but the problem still persists (2). There have been several reports on the triglycerides that crystallize when palm olein is kept at low temperatures (3–5). However, little is known about the really hard-melting glycerides that first form during cold storage of the oil. The complexity of the triglycerides makes the identification process even more difficult. This report describes a study on the identification of the triglycerides of the fat crystals that developed when palm olein is stored at 12.5°C, the usual temperature used to study the clouding point of palm olein.

*To whom correspondence should be addressed at the Department of Food Technology, Universiti Pertanian Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

MATERIALS AND METHODS

Malaysian unblended RBD palm olein (*Elaeis guineensis* var. Tenera) was obtained from three different refineries. All chemicals used were either of analytical or high-performance liquid chromatographic (HPLC) grade. Standard methyl esters and triglycerides were purchased from Sigma Chemical Company (St. Louis, MO).

Crystallization was carried out in standard 115-mL (4 oz) cloud point determination bottles (Beatson bottle) with a diameter of 42 mm and wall thickness of 2 mm, as described in American Oil Chemists' Society Official Method Cc 6-25 (6). Crystallization temperature was 12.5°C, and the crystals were collected from 12 to 24 h at 3-h intervals. A 50 ± 0.005 g oil sample was placed in each bottle, and the crystal fraction was collected after centrifugation at 3,000 rpm for 3 min at 12.5°C. The liquid portion was discarded without disturbing the crystals, which were retained in the bottom of the bottle. The crystals in the bottle were washed with 10–15 mL acetone of the same temperature.

The test methods adopted in this study were gas–liquid chromatography (GLC) for analyses of triglyceride carbon numbers (CN) (7) and fatty acid methyl esters (FAMEs) (7) as used by the Palm Oil Research Institute of Malaysia (8). A Pye Unicam (Cambridge, United Kingdom) Series 204 gas chromatograph system, equipped with flame-ionization detector and a Hewlett-Packard (Palo Alto, CA) data integrator (Model 3380A) was used for both CN and FAME analyses. For CN analysis, a glass column (0.5 m × 30 mm i.d.) packed with 3% OV-1 on Gas Chrom Q 100/120 mesh size (Supelco, Bellefonte, PA) was used. The nitrogen carrier gas flow rate was set at 80 mL/min, the detector and injector temperatures at 400°C, and the oven temperature was programmed from 280 to 350°C at a rate of 5°C/min. For FAME analysis, a glass column (1.8 m × 2.5 mm i.d.) packed with 10% SP 2330 on 100/120 Supelcoport (Supelco) was used. The nitrogen carrier gas flow rate was set at 40 mL/min, the detector and injector temperatures at 220°C, the isothermal column temperature at 190°C. HPLC method used to determine the triglyceride composition was modified from Dong's and Dicesare's method (9). The HPLC system used was equipped with a Waters (Milford, MA) HPLC pump (Model 501), Waters differential refractometer (Model 410), Waters system interface module, a column oven, a computer and a printer. A single commercially packed (250 mm × 4.0 mm) RP-18 (Merck, Darmstadt, Germany) column with a particle size of 5 μm was used. The mobile phase was a mixture of acetone/acetonitrile (63.5:36.5), and the flow rate was 1 mL/min. The injection volume was 10 μL. All analyses were done in replicates of four, and the results are expressed as mean weight percentage. Peaks were identified by injecting standard triglycerides and diglycerides individually and by matching retention time.

RESULTS AND DISCUSSION

CN. The CN composition of palm olein used in this research was C48 (3.9%), C50 (40.0%), C52 (45.6%) and C54 (10.5%). The composition was different from that of the crystals collected over the period of 12–24 h at 12.5°C. Table 1 shows the CN composition of the palm olein triglycerides stored at 12.5°C. The most significant increase in content was for triglycerides with a CN of 50. The concentration increased from 40.0% in the oil (control) to 44.5% at 18 h of storage. Beyond 18 h the concentration decreased, and at 24 h, the concentration of C50 triglycerides in the fat crystals was 41.3% ($P < 0.05$). The composition of C48 increased slightly from 3.9% (control) to 5.2% at 12 h and 4.8% at 18 h and decreased thereafter to values similar to the original sample ($P < 0.05$). As shown in Table 1, the concentration of C52 triglycerides decreased with increasing time of storage with the lowest concentration obtained in clouds collected at 18 h. The concentration then increased again to levels similar to those in the original oil sample ($P < 0.05$). Cloud formation also caused the concentration of C54 triglycerides to decrease between 12 h (9.9%) and 18 h (9.6%) when compared with the control (10.5%) ($P < 0.05$). In all of these cases, the concentration of the triglycerides in terms of CN approached that of the original oil sample as storage time progressed. This is due to the general solidification (80%) of the oil after several hours at 12.5°C.

GLC on an OV-1 column separates the triglyceride molecules according to the number of carbon atoms they possess. Therefore, tripalmitin (PPP) with three acyl chains of 16 carbon atoms will have a CN of 48 (abbreviated to C48). However, C48 could also be composed of MPS (myristic acid, C14:0; palmitic acid, C16:0; stearic acid, C18:0) or MPO (C14:0; C16:0; oleic acid, C18:1) or MPL (C14:0; C16:0; linoleic acid, C18:2) or their isomers. Likewise, C50 could be POP (C16:0; C18:1; C16:0) or PLP (C16:0; C18:2; C16:0) and their isomers. Therefore, CN values alone cannot be used to identify the actual glycerides that made up the clouds obtained between 15–18 h of storage.

FAME. The fatty acid composition of palm olein used in this study was myristic (C14:0) 0.8%, palmitic (C16:0) 38.6%, stearic (C18:0) 3.5%, oleic (C18:1) 43.8%, linoleic (C18:2) 12.6% and trace amount of lauric (C12:0) and arachidic (C20) acids. The changes in fatty acids during storage are presented in Table 2. Oleic and linoleic acids exhibited a significant decrease in concentration. Palmitic and stearic acids both exhibited an increase in value. The greatest change in concentration occurred for oleic and linoleic acids, which experienced a decrease of about 5.5 and 8.8%, respectively. Lauric and myristic acids, being minor constituents (less than 1%), did not show any significant changes. The highest concentrations achieved by both palmitic and stearic acids were 41.7 and 3.7% ($P < 0.05$), respectively, and occurred at 15 h of storage.

Therefore, medium-chainlength saturated acyl groups (lauryl, myristoyl) behaved like the unsaturated acyl groups (oleoyl, linoleoyl). The saturated fatty acids, palmitic and stearic acids, exhibited opposite behavior. Because palmitic and oleic acids are the major components of palm olein, it is to be expected that crystallization of the oil would depend on them heavily. The increase of palmitic acid helps to explain the early crystallization of triglycerides containing palmitic acid in their molecules during the storage period as compared to triglycerides containing higher proportions of unsaturated acyl groups. These triglycerides have considerably lower melting temperature due to the presence of unsaturated fatty acids (10).

Triglycerides. Based on the HPLC chromatogram of palm olein (Fig. 1), it can be estimated that there are 17 triglycerides found in palm olein. They are LaLaLa (0.08%); LaLaM (0.43%); MMLa (0.16%); MMM (0.59%); MPO (0.69%); MPL (3.01%); PPO (27.95%); PPL (10.65%); LLL (0.007); POS (5.12%); POO (27.49%); PLO (12.64%); OOS (3.82%); SOS (0.58%); SLS (0.20%); OOO (4.57%); and OOL (2.19%) (La, lauric; M, myristic; P, palmitic; O, oleic;

TABLE 1

Triglyceride Composition Based on Carbon Number During Storage of Palm Olein at 12.5°C (wt%)

	Triglyceride carbon number[a]			
Hours	C48	C50	C52	C54
Control	3.9^{c}	40.0^{e}	45.6^{b}	10.5^{b}
12	5.2^{b}	41.6^{d}	43.6^{c}	9.9^{cd}
15	3.7^{c}	44.0^{b}	41.9^{d}	10.2^{bcd}
18	4.8^{b}	44.5^{b}	41.1^{e}	9.6^{d}
21	3.0^{d}	43.2^{c}	43.5^{c}	10.4^{bc}
24	2.8^{d}	41.3^{d}	45.2^{b}	10.7^{b}
$LSD_{0.05}$	0.49	0.58	0.54	0.59

[a]Means of four readings. Means in a column followed by different letters are different ($P < 0.05$). LSD, least significant difference at $P < 0.05$.

TABLE 2

Fatty Acids Composition During Low-Temperature Storage (wt%)

	Fatty acids[a]						
Hours	C12	C14	C16	C18:0	C18:1	C18:2	C20
Control	trace[b]	0.8^{c}	38.6^{g}	3.5^{e}	43.8^{c}	12.6^{c}	trace
12	trace	0.8^{c}	40.2^{e}	3.6^{d}	42.6^{e}	12.1^{d}	trace
15	trace	0.8^{d}	41.7^{c}	3.7^{c}	41.5^{g}	11.7^{c}	trace
18	trace	0.7^{e}	41.5^{c}	3.5^{e}	42.0^{f}	11.6^{f}	trace
21	trace	0.7^{d}	40.5^{d}	3.6^{e}	42.6^{e}	11.9^{e}	trace
24	trace	0.7^{f}	39.5^{f}	3.4^{f}	43.2^{d}	12.5^{c}	trace
$LSD_{0.05}$	—	1.2×10^{-2}	0.25	7.1×10^{-2}	0.16	0.18	—

[a]Means of four readings. Means in a column followed by different letters are different ($P < 0.05$). LSD = least significant difference at $P < 0.05$.
[b]Less than 0.5%.

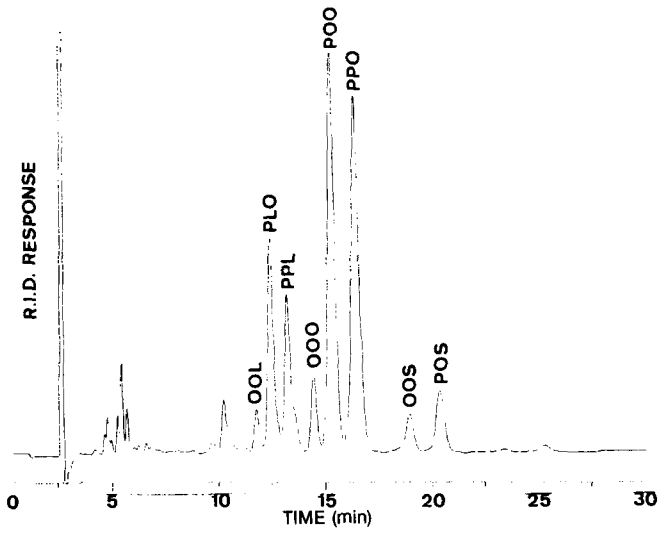

FIG. 1. Typical high-performance liquid chromatography chromatogram of palm olein (control). R.I.D., refractive index detector.

L, linoleic; S, stearic.) (Table 3). An increase was observed in PPO and POS, and a decline was found in the composition of POO, PLO, OOO, OOL and MPL during the first 18 h of crystallization. Figure 2 shows the chromatogram of crystals collected at 18 h. The rest of the components (LaLaLa, LaLaM, LLL, MMLa, MMM, MPO, PPL, OOS, SOS and SLS) did not show obvious changes.

It has been well accepted that crystallization behavior of triglycerides depends on the number of double bonds in their acyl chains and the number of carbon atoms in their hydrocarbon chains. The melting point of a triglyceride increases as the number of carbons in its hydrocarbon chains increases and decreases as the degree of unsaturation increases (10). Table 4 shows the composition of the triglycerides with respect to degree of unsaturation. The concentration of diunsaturated and triunsaturated triglycerides showed a decline, whereas monounsaturated exhibited an increase. Fully saturated and polyunsaturated (number of double bond > 3) triglycerides did not show an obvious change because they are minor components in palm olein.

The major components, POP and POO (27.95% and 27.49%), are to be watched closely as they will help us to understand the crystallization behavior of palm olein. POP showed an increase in concentration, and this agrees with the findings of the CN analysis of C50. POO and PLO exhibited a decrease, and it was supported by CN analysis of C52. However, POS, which is also a C52, showed an increase instead of a decrease. This could well be due to the presence of saturated fatty acids (palmitic and stearic) in its molecule. OOO, MPL and OOL decreased, as did POO and PLO, even though they stem from C54, C48 and C54, respectively. However, they all have two or more double bonds in their acyl chains of the triglyceride molecule, whereas POP and POS only have one. This indicates that the number of double bonds in the triglyceride molecule can significantly lower the melting point and, therefore, enhance the stability of the oil during low-temperature storage.

The lower melting points of triglycerides rich in unsaturated fatty acids are related to differences in three-

TABLE 3

Triglyceride Composition Based on High-Performance Liquid Chromatography Analysis During Low-Temperature Storage (wt%)

Hours	Triglyceride[a]															
	MPO	MPL	PPO	PPL	POS	POO	POL	OOS	SOS	SLS	OOO	LaLaLa	LaLaM	LLL	MMLa	MMM	OOL
Control	0.69^{abc}	3.01^{a}	27.95^{c}	10.65^{d}	5.12^{c}	27.49^{a}	12.64^{a}	3.28^{ab}	0.58^{b}	0.20^{c}	4.57^{a}	0.08^{a}	0.43^{b}	0.007^{ab}	0.16^{b}	0.59^{b}	2.19^{ab}
12	0.66^{d}	2.96^{a}	29.32^{b}	10.72^{cd}	5.42^{b}	26.42^{b}	12.31^{a}	3.36^{a}	0.62^{ab}	0.31^{a}	4.41^{a}	0.11^{a}	0.50^{a}	0.04^{ab}	0.17^{ab}	0.56^{b}	2.08^{bc}
15	0.66^{cd}	2.72^{b}	32.92^{a}	10.86^{bc}	6.04^{a}	24.05^{d}	11.17^{b}	2.69^{c}	0.67^{a}	0.20^{c}	4.07^{bc}	0.06^{ab}	0.10^{c}	0.03^{b}	0.167^{b}	0.52^{b}	1.9^{c}
18	0.68^{bcd}	2.69^{b}	33.53^{a}	10.95^{b}	6.04^{a}	23.98^{d}	11.13^{b}	3.04^{b}	0.67^{a}	0.28^{b}	3.98^{c}	0.08^{a}	0.10^{c}	0.009^{a}	0.195^{a}	0.52^{b}	1.89^{c}
21	0.72^{a}	3.14^{a}	29.82^{b}	11.15^{a}	5.29^{bc}	25.54^{c}	12.83^{a}	2.74^{c}	0.57^{b}	0.16^{d}	4.61^{a}	0.0^{b}	0.0^{d}	0.04^{ab}	0.16^{b}	0.70^{a}	2.39^{a}
24	0.70^{ab}	2.96^{a}	30.10^{b}	10.96^{b}	5.48^{b}	26.32^{b}	12.22^{a}	3.17^{ab}	0.62^{ab}	0.17^{d}	4.36^{ab}	0.0^{b}	0.07^{c}	0.04^{ab}	0.165^{b}	0.57^{b}	2.08^{bc}
$LSD_{0.05}$	2.96×10^{-2}	0.19	1.21	0.15	0.29	0.16	0.72	0.24	5.3×10^{-2}	1.16×10^{-2}	0.31	6.11×10^{-2}	3.70×10^{-2}	5.54×10^{-2}	2.64×10^{-2}	0.102	0.28

[a]La, lauric; M, myristic; P, palmitic; S, stearic; O, oleic; and L, linoleic acid. Other abbreviation as in Table 1. Means of four readings. Means in a column followed by different letters are different ($P < 0.05$).

TABLE 4

Triglyceride Composition with Respect to the Degree of Unsaturation (wt%)

Hours	Triglycerides				
	Saturated	Monounsaturated	Diunsaturated	Triunsaturated	Polyunsaturated
Control	1.3	34.3	44.6	17.2	2.3
12	1.3	36.0	43.8	16.7	2.1
15	0.8	40.3	40.5	15.2	1.9
18	0.9	41.0	40.9	15.1	2.0
21	0.9	36.4	42.7	17.4	2.4
24	0.8	36.9	43.6	16.6	2.1

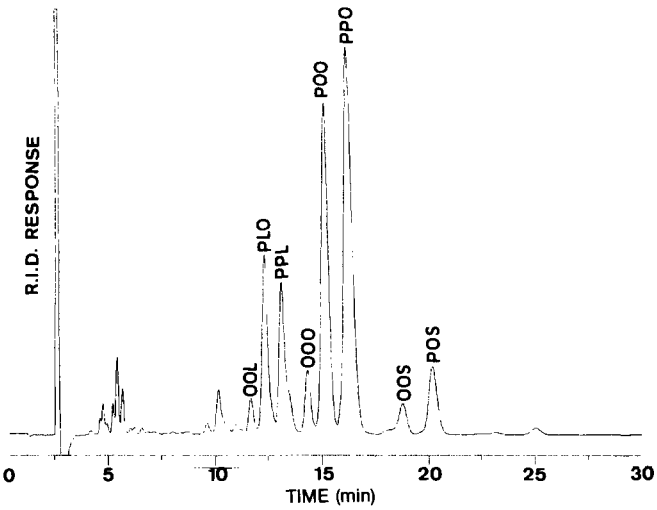

FIG. 2. Typical high-performance liquid chromatography chromatogram of palm olein at 12.5°C, 18 h. O, oleic; L, linoleic; P, palmitic; S, stearic. See Figure 1 for other abbreviation.

dimensional shape between the hydrocarbon chains of unsaturated and of saturated fatty acid components. In the saturated triglyceride molecule, the fatty acid hydrocarbon chains lie parallel to each other, and the molecule has an ordered, compact shape. Hence, dispersion forces between these hydrocarbon chains are strong. Because of this compact nature and the interaction by dispersion forces, triglycerides rich in saturated fatty acids have higher melting points (above room temperature). In the unsaturated triglyceride molecule, the *cis* double bond of fatty acids reduces the dispersion forces between them so that they cannot pack together as closely and compactly. Consequently, unsaturated triglycerides have lower melting points than triglycerides that are more highly saturated.

This study showed that the fat crystals of clouds are caused by triglycerides that possess saturated fatty acids. Based on the results obtained, the major components of palm olein clouds are PPO and POS, which were 23 and 18% higher, respectively, in clouds than in the mother oil. For palm olein to be able to withstand low-temperature storage, the high-melting glycerides should be removed from the oil or the oil palm must be custom-designed to produce more low-melting triglycerides. The former can be achieved by selective fractionation by using appropriate solvents and temperatures. The separation of triglyceride molecules according to the number of *cis* double bonds is possible by crystallizing in solvents containing $AgNO_3$ (11). However, crystallization of triglycerides from $AgNO_3$-saturated methanol/acetone (70:30) at −10°C produced a precipitation that was mainly composed of SSO and SSL, both of which are composed of one or two double-bond fatty acids in their triglyceride molecule (11). The potential of this technique to separate PPO and POS from POO could be realized by using various solvents and crystallization conditions. Therefore, further studies should be carried out.

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