Fractionation of Menhaden Oil and Partially Hydrogenated Menhaden Oil: Characterization of Triacylglycerol Fractions

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ABSTRACT: Menhaden oil (MO) and partially hydrogenated menhaden oil (PHMO) were dry-fractionated and solvent-fractionated from acetone. After conversion to fatty acid methyl esters, the compositional distribution of saturated, monounsaturated, *trans,* and n-3 polyunsaturated fatty acids (PUFA) in the isolated fractions was determined by gas chromatography. Acetone fractionation of MO at −38°C significantly increased the n-3 PUFA content in the liquid fractions over that of starting MO ($P < 0.05$). For PHMO, liquid fractions obtained by low-temperature crystallization (−38, −18, and 0°C) from acetone showed significant increases ($P < 0.05$) in monounsaturated fatty acid (MUFA) content over that of the starting PHMO. For selected MUFA-enriched fractions, reversed-phase high-performance liquid chromatography (HPLC) was used to separate, isolate, and characterize the major triacylglycerol (TAG) molecular species present. Thermal crystallization patterns for these fractions also were determined by differential scanning calorimetry (DSC). The results demonstrated that under the appropriate conditions it is possible to dry-fractionate or solvent-fractionate MO and PHMO into various solid and liquid fractions that are enriched in either saturated, monounsaturated, polyunsaturated, or the n-3 classes of fatty acids. Moreover, characterization of these TAG fractions by reversed-phase HPLC gives insight into the compositional nature of the TAG that are concentrated into the various fractions produced by these fractionation processes. Finally, the DSC crystallization patterns for the fractions in conjunction with their fatty acid compositional data allow for the optimization of the fractionation schemes developed in this study. This information allows for the production of specific TAG fractions from MO and PHMO that are potentially useful as functional lipid products.

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KEY WORDS: Dry fractionation, DSC, menhaden oil, partially hydrogenated menhaden oil, reversed-phase high-performance liquid chromatography, solvent crystallization.

Aside from their nutritional role in the human diet, the various fatty acid classes are purported to have different roles in regard to their health effects (1). In general, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are thought to have desirable effects on the causative factors regulating coronary artery disease. For example, the beneficial health effects of the n-3 class of PUFA, such as 5,8,11,14,17-eicosapentaenoic acid (EPA) and 4,7,10,13,16,19-docosahexaenoic acid (DHA), are well documented, whereas large amounts of saturated fatty acids (SFA) and *trans*-fatty acids are generally regarded as undesirable in the human diet (2,3).

Dry fractional crystallization is a thermomechanical separation process wherein triacylglycerol (TAG) species with the highest melting points preferentially crystallize during cooling from the neat liquid oil or melted fat. After crystallization is complete, the solid phase is separated from the liquid phase by one of several physical processes (4,5). Alternatively, solvent (typically acetone) crystallization is used for promoting TAG crystal formation, because TAG at low temperature generally form more stable crystals with solvent than without solvent (6). Accordingly, TAG species with a higher (or lower) level of desirable (or undesirable) fatty acids can be separated by both fractional crystallization processes for use in nutritional or functional food applications. Examples of such processes include the fractionation of lauric acid from coconut oil and palm kernel oil, and γ-linolenic acid from fungal oils (6,7).

The objectives of this study were to prepare TAG fractions rich in either PUFA or MUFA from menhaden oil (MO) and partially hydrogenated menhaden oil (PHMO) by dry fractionation or solvent fractionation. To determine if the fractionation processes achieved the stated goals, the SFA, MUFA, PUFA, *trans*, and n-3 PUFA fatty acid contents of the fractions obtained were determined. The oil fractions also were analyzed by differential scanning calorimetry (DSC) to determine their crystallization patterns. Finally, fractions high in MUFA were separated by reversed-phase high-performance liquid chromatography (HPLC) and selected TAG molecular species were isolated and their fatty acid composition determined.

EXPERIMENTAL PROCEDURES

Materials. MO and 14% boron trifluoride (BF_3) in methanol were obtained from Sigma Chemical Company (St. Louis, MO). PHMO was a gift from Omega Protein (Reedville, VA). Acetone, analytical grade, was obtained from Baxter Health Corp. (Muskegon, MI).

Fatty acid methyl ester (FAME) analysis. TAG samples (20 mg) were reacted with 2 mL of 14% BF_3 in methanol at 60°C for 15 min. After cooling on ice, 1 mL of saturated NaCl solution and 2 mL of isooctane were added and the mixture was vortexed. The isooctane layer containing the FAME was removed and dried over anhydrous sodium sulfate and analyzed by gas chromatography (GC). A Hewlett-Packard (Agilent,

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Wilmington, DE) Model 5890 Series II GC equipped with an automatic split injector and flame-ionization detector was used for FAME analysis. The methyl esters were separated on a cross-linked polyethylene glycol column (HP-INNOWAX, 30 m × 0.53 mm i.d., 0.25 µm film thickness). Helium was the carrier gas at a flow of 5.5 mL/min. The column oven conditions were as follows: 120°C, held for 2 min, heated to 230°C at 5°C /min, and held at final temperature for 22 min. The injector and detector temperatures were 260°C.

Reversed-phase HPLC analysis. TAG fractions were analyzed by nonaqueous reversed-phase HPLC on a Hewlett-Packard Model 1050 HPLC equipped with a Beckman/Altex Ultrasphere ODS 5 µm (4.6 mm × 25 cm) column and a Varex (Burtonville, MD) ELSD II mass detector. Estimations of partition numbers (PN) for individual TAG peaks in the fractions were made by comparison with a regression model constructed for PN vs. retention time for a standard TAG mix (G-1) obtained from Nu-Chek-Prep (Elysian, MN), by Foglia, *et al.* (8). Solvent gradient and other HPLC conditions used were as described (8). The chromatograms of selected samples were divided into four retention time segments based on PN, and the eluant for each segment was isolated manually from the column for subsequent FAME analysis by insertion of a proportioning valve between the HPLC column and the detector.

Fourier transform infrared (FTIR) spectroscopy. A Nicolet Impact 400D (Nicolet Instrument Inc., Madison, WI) FTIR spectrometer with Omnic operating software was used to measure *trans*-fatty acid content of the TAG fractions. The instrument was purged with nitrogen (0.4 psi), and sodium chloride cells (25 mm i.d × 4 mm thickness) were used for analysis. All spectra were recorded after 25 scans at a resolution of 1 cm^{-1}. For calibration, a reference background spectrum was taken for the clean empty cell, which was subtracted automatically from each sample spectrum. Standard mixtures of methyl elaidate (5–60 wt%) in methyl oleate were prepared for construction of a *trans*-fatty acid standard curve. For *trans* acid analysis, each standard mix (about 30 mg) was dissolved in chloroform (1.2 mL), and approximately 40 µL of the solution was applied onto the infrared (IR) cells and solvent was evaporated with nitrogen for 5 min. TAG fractions, after conversion to FAME and after removal of solvent, were analyzed similarly for their *trans*-fatty acid content (9).

Differential scanning calorimetry (DSC). Melting profiles for MO, PHMO, and their fractions were obtained by DSC on a PerkinElmer (PerkinElmer Corp., Norwalk, CT) Model Pyris 1. Samples were heated to 80°C, and after 10 min at this temperature the cooling curve was obtained by cooling at 10°C/min until reaching –60°C (10).

Dry fractionation. Approximately 5 g of MO or PHMO was placed into a 50-mL polypropylene centrifuge tube and fractionally crystallized at various temperatures for 24 h. After centrifugation (7,600 × *g*, 10 min) in a centrifuge adjusted to the fractionation temperature, the liquid phase was decanted from the solid phase. Before fractionation, MO and PHMO were held at 60°C for 5 min and at 80°C for 10 min, respectively, to remove memory effects of polymorphic TAG forms that may have been present originally.

Solvent fractionation. Approximately 2 g of MO or PHMO was placed into a 50-mL polypropylene centrifuge tube and fractionally crystallized from acetone. The solute to solvent ratio used was 1:20 (wt/vol) and crystallization was conducted at three temperatures (−38, −18, and 0°C). Other solute to solvent ratios (1:5 and 1:10) and temperatures (−10 and 5°C) were studied, but the results were not significantly different from those listed in Table 1. All solvent crystallization experiments were held at the specified temperature for 24 h. After crystallization was complete, each centrifuge tube was placed into an insulated 250-mL wide-mouth centrifuge bottle to minimize temperature changes during centrifugation. Decanting the liquid phase from the crystal pellet after centrifugation in a prechilled centrifuge (2,100 × *g*, 10 min) separated the liquid and solid phases. Acetone was removed from the fractions by evaporation under a stream of nitrogen at 60°C until constant weight was obtained.

Statistics. The statistical comparisons were made according to Statistical Analysis System (SAS, Cary, NC) (1996). A Bonferroni (Dunn) *t*-test was performed on the means of values for fatty acid groupings. The tested significance level was $P < 0.05$ (11).

RESULTS AND DISCUSSION

The summed (∑) wt% fatty acid class profiles (∑ SFA, ∑ MUFA, ∑ PUFA, and ∑ EPA + DHA) for MO (Table 1, entry 1) and PHMO (Table 1, entry 12) are listed in Table 1. Similarly listed are the fatty acid class profiles for the solid and liquid fractions obtained by dry fractionation of MO at –10, –5, and 0°C (Table 1, entries 2–7) and PHMO at 18 and 30°C (Table 1, entries 13–16). MO did not give an isolatable (<2%) solid fraction above 0°C or liquid fraction below −10°C. Similarly, PHMO remained mostly liquid above 31°C and solid below 18°C. Dry fractionation of PHMO also was studied between 18 and 30°C at 3°C intervals (data not shown). In general, however, changes in the ∑ SFA, ∑ MUFA, ∑ PUFA, and ∑ EPA + DHA classes for the liquid and solid fractions produced were not significantly ($P < 0.05$) different from those of the starting MO or PHMO. Both MO and PHMO are complex mixtures of TAG species that contain numerous fatty acids of varying carbon chain length and degrees of unsaturation. Because of their complex nature, the crystallization of individual TAG species from MO and PHMO occurs over a wide temperature range, making it difficult to concentrate the TAG classes present in these oils by this process.

In general, the separation of higher-melting TAG from complex mixtures of TAG is facilitated when low-temperature solvent fractionation is used. This is so because TAG molecules form more stable crystals within shorter time periods when they are fractionally crystallized from a solvent. Although the use of a solvent adds additional costs to the fractionation process, solvent fractionation has been used to prepare specialty fats (4). Among solvents, acetone is considered

TABLE 1
Summed (•) Distribution of Fatty Acid Classes for Fractions Produced by Either Dry or Wet (solvent: acetone) Fractionation of Menhaden Oil (MO) and Partially Hydrogenated Menhaden Oil (PHMO)[a]

Entry		∑ SFA	∑ MUFA	∑ PUFA	∑ EPA + DHA	% *trans*	Wt%
1	MO	35.1[h]	24.3[e]	10.2[e,f]	30.4[f,g]	—	—
2	MO −10L[b]	32.3[h,i]	25.4[e,f,g]	13.0[e]	29.3[e,f]	—	10.8
3	MO −10S[b]	37.4[g]	24.4[e,f,g]	10.4[f,g]	27.8[f,g]	—	89.2
4	MO −5L[b]	31.9[i]	24.5[e,f,g]	11.3[e]	32.3[e,f]	—	53.7
5	MO −5S[b]	39.0[g]	22.1[f,g,h]	10.2[e,f]	28.7[f,g]	—	46.3
6	MO 0L[b]	34.0[h,i]	23.9[e,f,g]	10.9[e,f]	31.2[e,f,g]	—	67.0
7	MO 0S[b]	38.6[g]	22.1[f,g,h]	10.6[e,f]	28.7[f,g]	—	33.0
8	MO −38L[c]	27.3[j]	26.3[e]	11.1[e,f]	35.3[e]	—	62.2
9	MO −38S[c]	46.4[f]	21.3[g,h]	8.7[f]	23.6[h]	—	37.8
10	MO −18L[c]	32.5[h,i]	24.4[e,f]	11.2[e,f]	31.9[e,f]	—	86.3
11	MO −18S[c]	55.3[e]	18.9[h]	7.4[f]	18.4[h]	—	13.7
12	PHMO	49.7[g]	47.1[h,i]	3.2[e,f]	—	33.1[e,f]	—
13	PHMO 30L[b]	49.1[g]	46.0[h,i]	4.8[e]	—	31.8[e,f]	89.2
14	PHMO 30S[b]	53.4[e,f]	43.0[i,j]	3.6[e,f]	—	32.0[e,f]	10.8
15	PHMO 18L[b]	46.6[g]	49.8[g,h]	3.6[e,f]	—	30.5[f]	22.7
16	PHMO 18S[b]	52.1[f,g]	45.0[h,i]	2.9[f]	—	33.7[e,f]	77.3
17	PHMO −38L[c]	34.5[i]	58.4[e]	3.3[e,f]	—	31.2[e,f]	33.8
18	PHMO −38S[c]	54.2[e,f]	43.1[i,j]	2.7[f]	—	33.3[e,f]	66.2
19	PHMO −18L[c]	40.5[h]	55.1[e,f]	4.5[e]	—	35.8[e]	49.5
20	PHMO −18S[c]	57.3[e]	39.9[j]	2.8[f]	—	36.0[e]	50.5
21	PHMO 0L[c]	40.8[h]	51.6[f,g]	3.5[e,f]	—	34.9[e,f]	67.5
22	PHMO 0S[c]	53.4[f,g]	37.1[j]	2.4[f]	—	34.0[e,f]	32.5

[a]Fatty acid composition determined by gas chromatography (GC) (area %); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; and DHA, docosahexaenoic acid. Weight percent (wt%) recovery is defined as the sum of liquid and solid fractions, e.g., MO-5L + MO-5S = 100%. Mean values within a column having the same roman superscript (e–j) do not differ significantly ($P < 0.05$). The analyses are for entries 1–11 and 12–22, respectively, for MO and PHMO.
[b]Dry fractionation carried out at 0, −5, and −10°C for MO and at 18 and 30°C for PHMO.
[c]Solvent (acetone) fractionation of MO and PHMO carried out at 0, −18, and −38°C. No crystallization of MO occurred at temperatures ≥0°C.

as one of the more suitable for promoting stable TAG crystal formation (7). Currently, the maximum permissible residue level for acetone is 30 ppm (Code of Federal Regulations 21CFR173.210). Accordingly, in this study, MO was solvent-fractionated from acetone at low temperatures (−38, −18, and 0°C). For the −38°C liquid fraction, the ∑ EPA + DHA content increased ($P < 0.05$) to 35.3% compared to the 30.4% in neat MO, and the yield recovery of the fraction was 62 wt% (Table 1, entry 8). For the −18 and 0°C liquid fractions the ∑ EPA + DHA content in these fractions was similar to that of MO. On the other hand, the ∑ MUFA and ∑ PUFA in the liquid fraction at −38°C and the ∑ MUFA, ∑ PUFA, and ∑ EPA + DHA in the liquid fraction at −18 and −0°C were not significantly different from that of MO. However, the −38 and −18°C solid fractions (Table 1, entries 9 and 11) had significant increases in ∑ SFA, but their wt% recovery was small, especially for the −18°C solid fraction. Apparently, the solvent fractionation temperatures and solvent ratio used in this study did not allow for the selective fractionation of the MUFA, PUFA containing TAG from MO.

Acetone fractionation of PHMO resulted in a significant increase in the ∑ MUFA in the liquid fractions obtained at the three temperatures studied (Table 1, entries 17, 19, and 21). Compared to neat PHMO, the ∑ MUFA in the liquid fractions increased with decreasing fractionation temperature by 10 to 24% and the wt% recovery of the fractions ranged from 34 to 68%. Surprisingly, when the fractionation temperature was decreased from 0 to −38°C, the ∑ MUFA in liquid fraction increased by 12% even though the wt% recovery decreased by half. As expected, as the ∑ MUFA increased significantly the ∑ SFA decreased significantly in the liquid fractions from PHMO. Conversely, for the solid fractions, significant increases in ∑ SFA were accompanied by significant decreases in ∑ MUFA (Table 1, entries 18, 20, and 22).

The hardening of oils by hydrogenation of the unsaturated fatty acids in fats and oils has been an important industry practice since the early 1900s (4). Hydrogenation, however, also induces isomerization of the *cis* unsaturated fatty acids to *trans* isomers, which are not commonly present in natural fats and oils. In general, it is suggested that the dietary intake of *trans*-fatty acids should be limited (12). Accordingly, to answer the question as to whether dry or solvent fractionation processes could produce fractions of lower *trans*-fatty acid content from PHMO, we measured the percent *trans*-fatty acid content for PHMO and the various fractions obtained from it (Table 1). For neat PHMO, a 33.1% *trans*-fatty acid

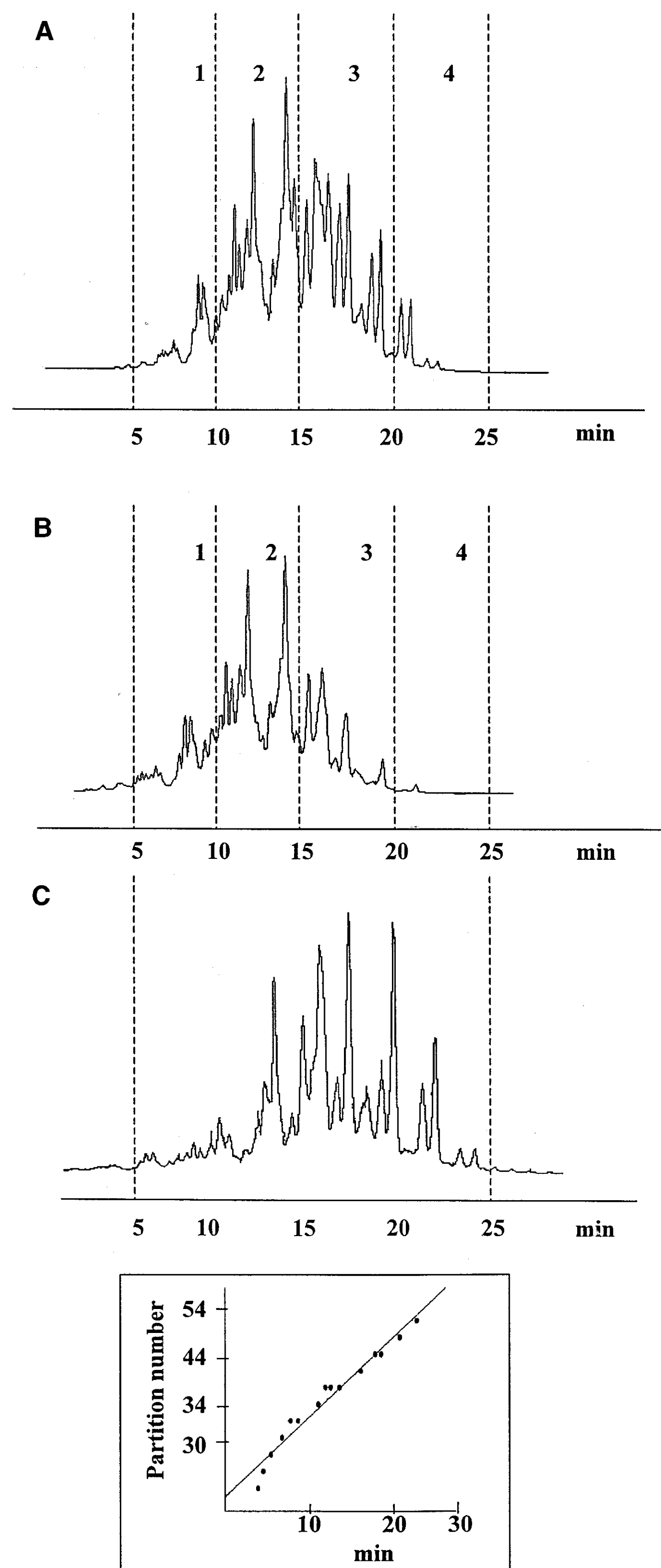

FIG. 1. Reversed-phase high-performance liquid chromatography (HPLC) chromatograms for (A) menhaden oil (MO), (B) liquid, and (C) solid fractions obtained from MO at −38°C. Chromatograms A and B are divided into four segments based on partition number (PN). Insert: Regression plot of triacylglycerol PN vs. retention time for standard triacylglycerol mixture.

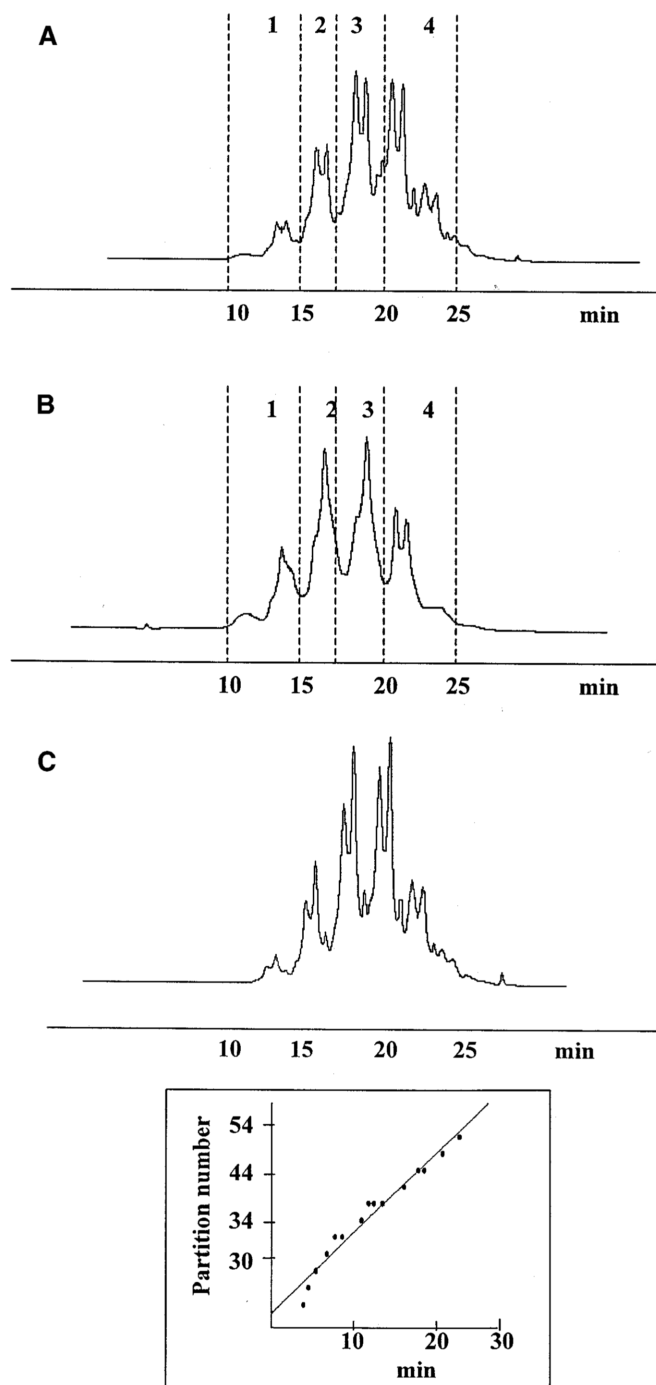

FIG. 2. Reversed-phase HPLC chromatograms for (A) partially hydrogenated menhaden oil (PHMO), (B) liquid, and (C) solid fractions obtained from PHMO by acetone crystallization at −38°C. Chromatograms A and B are divided into four segments based on PN. Insert: Regression plot of triacylglycerol PN vs. retention times for standard triacylglycerol mixture. For abbreviations see Figure 1.

content was determined (Table 1, entry 12). In the solid and liquid fractions, the *trans*-fatty acid content ranged between 30.3 and 36.0%, values not statistically different from PHMO itself, which indicated that the *trans*-fatty acid-containing TAG were not fractionated from *cis*-fatty acid-containing

TAG by the processes used in this study.

MO, PHMO, and the liquid and solid fractions obtained from them by solvent fractionation at −38°C were analyzed by nonaqueous reversed-phase HPLC. The HPLC separations obtained are presented in Figures 1 and 2. Reversed-phase HPLC, which separates TAG molecular species according to carbon chain length and number of double bonds of the acyl moieties that constitute the TAG, is widely used in the analysis of fats and oils (13). The HPLC chromatogram for MO (Fig. 1A) showed a poor resolution of TAG molecular species because MO is a complex mixture of TAG composed of fatty acids with carbon chains from C14 to C24, which may contain from zero to six double bonds. The chromatogram, however, can be segmented into regions of TAG PN based on the retention times of TAG standards. Accordingly, the HPLC chromatograms of MO and its −38°C liquid and solid fractions were divided into four retention time segments (Fig. 1). Each segment corresponded to TAG with the following range of PN: segment 1 (PN: 32–38), segment 2 (PN: 38–42), segment 3 (PN: 42–48), and segment 4 (PN: >48). In the HPLC chromatogram of MO, TAG with PN 38–42 and PN 42–48 (Fig. 1A, segments 2 and 3) accounted for 43 and 41% of the TAG in MO while only 7% of the TAG had PN > 48. TAG in the −38°C liquid fraction from MO (Fig. 1B) showed a similar distribution of TAG until PN 42 (Fig. 1B, segment 2); thereafter, several TAG peaks, assumed to be TAG containing more SFA, were reduced or eliminated. About 36% of the TAG in this fraction had PN > 42 whereas for MO they accounted for 48% of the TAG. In contrast, the chromatogram of the −38°C solid fraction (Fig. 1C) indicated that about 77% of the TAG in the solid fraction had PN > 42.

The HPLC segments for MO and its −38°C liquid fraction were isolated and analyzed by GC to determine their fatty acid composition (Table 2). The tabulated data are intended for qualitative comparison only. The individual fatty acid composition and summed (∑) fatty acid classes for the MO-TAG in segment 1 (MO_1) were 28.4% ∑ SFA, 17.9% ∑ MUFA, 17.6% ∑ PUFA, and 36.1% ∑ EPA + DHA. This suggests that most TAG species in this segment might contain one EPA or DHA moiety. In segment 4 (MO_4), the fatty acid class distribution was 41% ∑ SFA, 27% ∑ MUFA, 26% ∑ PUFA, and 6% ∑ EPA + DHA, which suggests that EPA or DHA acyl residues are not present in most TAG species. On the other hand, segment 4 (MOL_4) of the −38°C liquid fraction from MO was composed of 30% ∑ SFA, 42% ∑ MUFA, and 28% ∑ PUFA, suggesting that TAG species in this segment for the most part are composed of one saturated and two unsaturated (MUFA or PUFA) acyl residues. From Table 2, we conclude that ∑ MUFA content was significantly enriched in TAG molecules with PN 32–38 (MOL_1) and in TAG molecules with PN > 48 (MOL_4) of the −38°C liquid fraction from MO.

Similarly, Figure 2 shows the HPLC chromatogram for PHMO and the −38°C liquid and solid fractions obtained from it. As for MO, the chromatograms were divided into four retention time segments identified as $PHMO_1$ and $PHMOL_1$ (PN: ≤42), $PHMO_2$ and $PHMOL_2$ (PN: 44–46), $PHMO_3$ and $PHMOL_3$ (PN: 48–50), and $PHMO_4$ and $PHMOL_4$ (PN > 50), respectively. For PHMO (Fig. 2A), TAG species with PN ≥ 48 account for about 77% of the TAG and only 3.8% of TAG species have PN ≤ 42. For the −38°C liquid fraction from PHMO (Fig. 2B), however, TAG species with PN ≥ 48 accounted for about 54% of TAG and TAG species with PN ≤ 42 increased to 14% of TAG. In the −38°C solid fraction (Fig. 2C) the bulk of the TAG in this fraction eluted in segment 4 (PN: >50). For the PHMO-TAG segments (Table 3), the MUFA content ranged from 27 ($PHMO_3$) to 43% ($PHMO_2$). However, the MUFA content for the HPLC segments of the −38°C liquid fraction from PHMO varied from 42 ($PHMOL_1$) to 59% ($PHMOL_{3\&4}$) (Table 3). The foregoing indicated that in addition to carbon chain length the degree of unsaturation also affected the fractionation of TAG present in the PHMO.

The solid fat content (SFC) curves for MO and PHMO and the solid and liquid fractions obtained from them by acetone fractionation at −38°C are shown in Figure 3. MO and its −38°C solid fraction have 50% SFC at −11.4 and −7.5°C, respectively, while the −38°C MO liquid fraction has

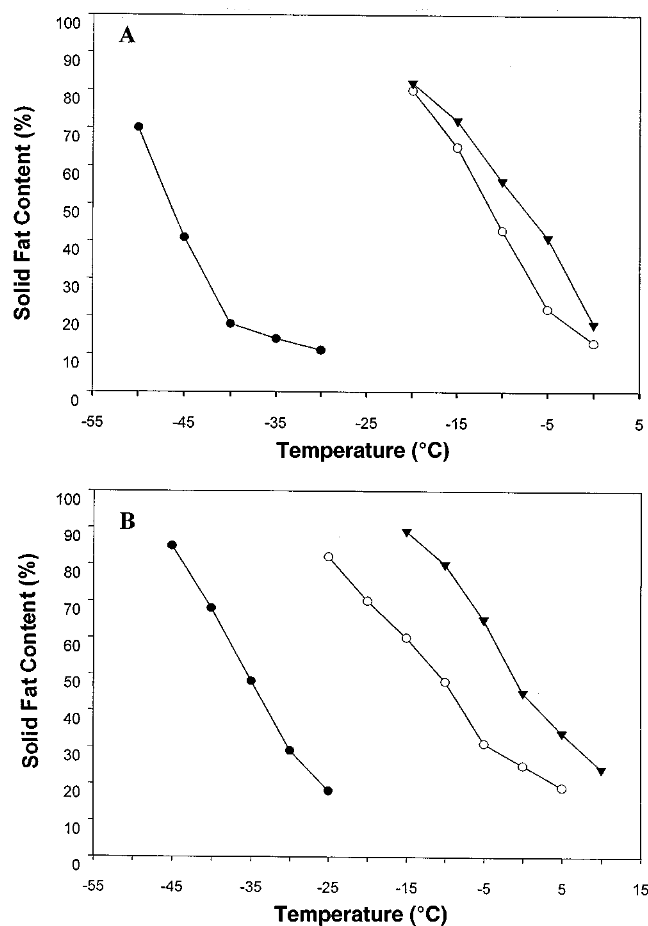

FIG. 3. Differential scanning calorimetry (DSC) solid fat content (%) curves for: (A) MO (○) and the liquid (●) and solid fractions (▲) obtained from MO by acetone fractionation at −38°C; and (B) PHMO (○) and the liquid (●) and solid (▲) fractions obtained from PHMO by acetone fractionation at −38°C. For abbreviations see Figures 1 and 2.

TABLE 2
Fatty Acid Composition of MO, –38¡C Liquid Fraction from MO (MOL), and Their Segmented Reversed-Phase High-Performance Liquid Chromatography (HPLC) Fractions[a,b]

Fatty acid	MO	MO_1	MO_2	MO_3	MO_4	MOL	MOL_1	MOL_2	MOL_3	MOL_4
C14:0	10.7^{d}	7.4^{e}	$9.8^{d,e}$	11.4^{d}	6.6^{f}	9.2^{d}	4.4^{f}	$7.7^{d,e}$	6.9^{e}	2.8^{f}
C14:1	0.4^{e}	0.8^{d}	0.4^{e}	0.3^{e}	0.7^{d}	0.7^{d}	0.4^{e}	0.8^{d}	$0.6^{d,e}$	—
C16:0	20.6^{e}	7.6^{g}	14.4^{f}	26.7^{d}	25.0^{d}	15.5^{e}	18.4^{d}	15.9^{e}	18.7^{d}	15.8^{e}
C16:1	14.1^{d}	10.3^{e}	11.6^{e}	14.9^{d}	10.0^{e}	16.4^{d}	5.7^{f}	10.9^{e}	16.4^{d}	8.6^{e}
C18:0	3.8^{g}	13.4^{d}	3.6^{g}	5.3^{f}	9.3^{e}	2.6^{g}	17.5^{d}	5.8^{f}	$3.9^{f,g}$	11.1^{e}
C18:1	9.8^{e}	6.8^{f}	8.3^{e}	11.1^{e}	15.9^{d}	9.2^{g}	27.3^{e}	7.2^{g}	11.1^{f}	33.1^{d}
C18:2	2.2^{g}	10.4^{e}	6.6^{f}	4.1^{g}	14.4^{d}	1.9^{g}	10.0^{e}	10.8^{e}	8.7^{f}	21.0^{d}
C18:3	2.5^{f}	7.2^{e}	6.5^{e}	2.5^{f}	12.0^{d}	1.3^{f}	6.1^{e}	8.2^{d}	5.5^{e}	$7.4^{d,e}$
C20:4	2.0	—	1.3	1.2	—	3.7^{d}	—	1.2^{e}	1.5^{e}	—
C20:5	$19.2^{e,f}$	24.6^{d}	$22.1^{d,e}$	11.8^{f}	6.1^{g}	22.6^{d}	5.6^{g}	16.9^{e}	12.2^{f}	—
C22:5	3.5	—	3.5	2.8	—	4.2^{d}	—	2.4^{e}	3.1^{e}	—
C22:6	11.2^{d}	11.5^{d}	11.9^{d}	7.9^{e}	—	12.7^{d}	4.6^{e}	12.2^{d}	11.3^{d}	—
∑ SFA	35.1^{e}	28.4^{f}	27.8^{f}	43.4^{d}	40.9^{d}	27.3^{e}	40.3^{d}	29.4^{e}	29.5^{e}	29.7^{e}
∑ MUFA	24.3^{d}	17.9^{f}	20.3^{e}	26.3^{d}	26.6^{d}	26.3^{f}	33.4^{e}	18.9^{g}	28.1^{f}	41.7^{d}
∑ PUFA	10.2^{f}	17.6^{e}	17.9^{e}	10.6^{f}	26.4^{d}	11.1^{g}	16.1^{f}	22.6^{e}	$18.8^{e,f}$	28.4^{d}
∑ EPA + DHA	30.4^{e}	36.1^{d}	$34.0^{d,e}$	19.7^{f}	6.1^{g}	35.3^{d}	10.2^{f}	$29.1^{d,e}$	23.5^{e}	—

[a]Liquid fraction obtained by crystallization from acetone at –38°C. Fatty acid composition determined by GC (area %): $MO_{1–4}$ and $MOL_{1–4}$ represent the isolated MO and MOL triacylglycerol segments shown in Figure 1.
[b]Area percent fatty acid as determined by GC. Mean values for MO–MO_4 and MOL–MOL_4 within the same row having the same roman superscript (d–g) are not significantly different ($P < 0.05$). For other abbreviations see Table 1.

TABLE 3
Fatty Acid Composition of PHMO, —38¡C Liquid Fraction from PHMO (PHMOL), and Their Segmented Reversed-Phase HPLC Fractions[a,b]

Fatty acid	PHMO	$PHMO_1$	$PHMO_2$	$PHMO_3$	$PHMO_4$	PHMOL	$PHMOL_1$	$PHMOL_2$	$PHMOL_3$	$PHMOL_4$
C14:0	12.5^{d}	10.9^{e}	$9.1^{e,f}$	6.3^{f}	5.9^{f}	11.3^{d}	11.5^{d}	9.0^{e}	5.5^{f}	4.2^{f}
C16:0	$29.1^{d,e}$	32.2^{d}	$29.3^{d,e}$	25.2^{e}	35.8^{d}	20.7^{e}	15.4^{f}	$23.0^{d,e}$	25.9^{d}	$22.1^{d,e}$
C16:1	21.3^{d}	13.4^{e}	13.8^{e}	7.5^{f}	7.6^{f}	29.0^{d}	$25.9^{d,e}$	21.2^{e}	10.5^{f}	8.5^{c}
C18:0	5.1^{f}	17.3^{e}	14.0^{e}	38.6^{d}	13.4^{e}	3.1^{g}	30.9^{d}	$7.0^{f,g}$	16.9^{e}	$11.1^{e,f}$
C18:1	21.7^{e}	23.4^{e}	23.9^{e}	13.3^{f}	27.8^{d}	26.4^{e}	16.3^{f}	32.4^{d}	33.0^{d}	37.1^{d}
C20:0	0.6^{f}	2.8^{e}	4.6^{d}	$1.5^{e,f}$	4.1^{d}	0.3^{f}	—	2.8^{e}	2.7^{e}	3.6^{d}
C20:1	7.5^{d}	—	5.3^{e}	1.3^{f}	$3.1^{e,f}$	6.4^{e}	—	4.1^{f}	$5.1^{e,f}$	13.2^{d}
C22:0	0.2^{f}	—	—	1.0^{e}	2.3^{d}	0.3	—	—	—	0.2
C22:1	2.0	—	—	5.1	—	2.5^{d}	—	0.5^{e}	0.4^{e}	—
∑ SFA	47.5^{f}	63.2^{e}	$57.0^{e,f}$	72.6^{d}	61.5^{e}	35.7^{f}	57.8^{d}	41.8^{e}	$51.0^{d,e}$	41.2^{e}
∑ MUFA	52.5^{d}	36.8^{f}	43.0^{e}	27.2^{g}	38.5^{f}	64.3^{d}	42.2^{f}	58.2^{d}	49.0^{e}	58.8^{d}

[a]Liquid fraction obtained by crystallization from acetone at –38°C. $PHMO_{1–4}$ and $PHMOL_{1–4}$ values represent the isolated PHMO and PHMOL triacylglycerol HPLC segments shown in Figure 2.
[b]Area percent fatty acid as determined by GC. Mean values for PHMO–$PHMO_4$ and PHMOL–$PHMOL_4$ within the same row having the same roman superscript (d–g) are not significantly different ($P < 0.05$). For other abbreviations see Tables 1 and 2.

50% SFC at −46.5°C (Fig. 3A). For PHMO and its −38°C solid fraction the 50% SFC are at −10.3 and −1.5°C, respectively, while the corresponding liquid fraction has 50% SFC at −35.4°C (Fig. 3B). As anticipated, the SFC data show that acetone fractionation of both MO and PHMO concentrated the higher- and lower-melting TAG molecular species in the oils into the solid and liquid fractions, respectively.

The DSC cooling curve for MO showed a crystallization onset temperature (T_C) (14) at 3.2°C and a broad low-temperature T_C at −4.3°C (Fig 4A). In contrast, for the −38°C MO solid fraction, the DSC cooling curve (Fig. 4B) showed only one exotherm peak with a sharp T_C at 1.5°C, which, like the SFC observation, suggested that the higher-melting TAG species present in MO were preferentially concentrated (co-crystallized) into this fraction. The DSC cooling curve for the −38°C MO liquid fraction had two exothermic peaks at approximately −24 and −40°C, which did not, however, have distinctly defined T_C values (Fig. 4C). The DSC crystallization curve for PHMO also showed two distinct exotherm peaks, one with a T_C of 9.8°C and a lower-temperature exotherm with T_C at –3.4°C (Fig. 5A). Similarly, the cooling curve for the −38°C PHMO solid fraction had a sharp exotherm peak with T_C at 13.7°C and a lower-temperature exotherm with T_C at 0.2°C (Fig. 5B), which suggested that co-crystallization of high-melting TAG species had not occurred. On the other hand, the cooling curve for the −38°C

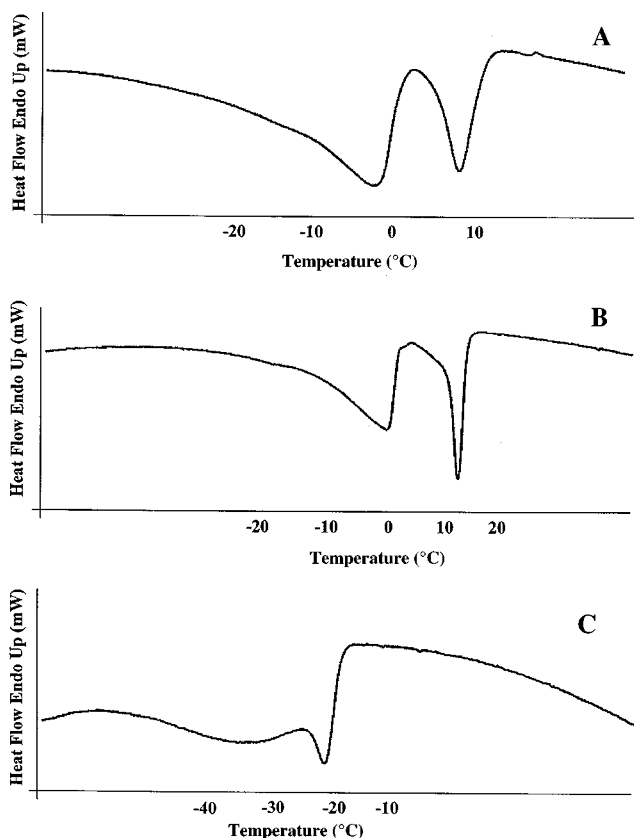

FIG. 4. DSC cooling curves (exotherm) for (A) MO, (B) solid fraction, and (C) liquid fraction obtained from MO by acetone fractionation at −38°C. For abbreviations see Figures 1 and 3.

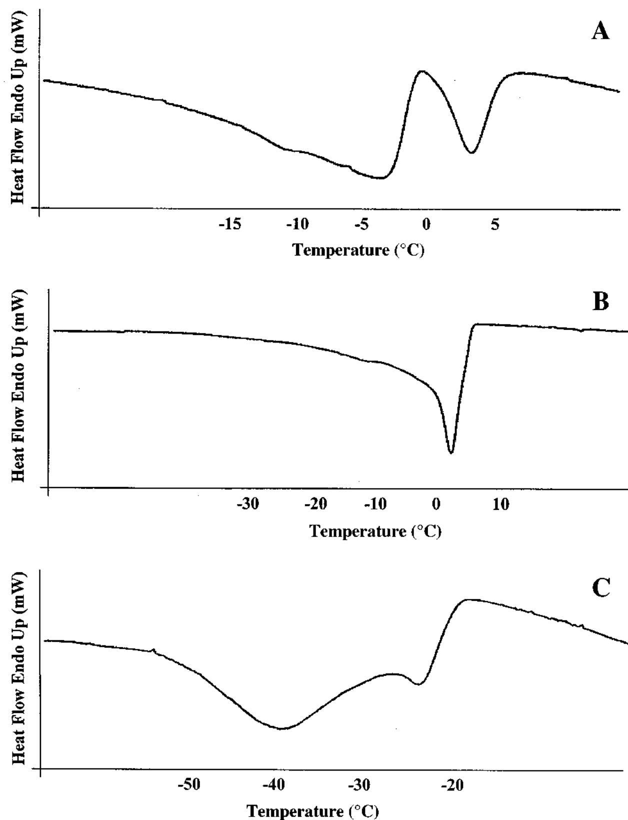

FIG. 5. DSC cooling curves (exotherm) for (A) PHMO, (B) solid fraction, and (C) liquid fraction obtained from PHMO by acetone fractionation at −38°C. For abbreviations see Figures 2 and 3.

PHMO liquid fraction showed a T_C of −22°C, and a broad indistinct exotherm around −22°C (Fig. 5C).

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