Influence of Lipase-Catalyzed Interesterification on the Oxidative Stability of Melon Seed Oil Triacylglycerols

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ABSTRACT: Melon seeds are rich in oil. However, the stability of melon seed oil (MSO) is low because of its high content of the essential fatty acid, linoleic acid (18:2n-6). MSO was physically blended or enzymatically interesterified with high-oleic sunflower oil (HOSO). The fatty acid composition of MSO was remarkably changed after interesterification. Palmitic (16:0), stearic (18:0), and oleic (18:1n-9) acid contents increased at the *sn*-2 position of triacylglycerols, whereas 18:2n-6 decreased due to interesterification. The oxidative stability of the physical and *Pseudomonas* sp. (PS30) lipase-interesterified blends was assessed with the Oxidative Stability Instrument, peroxide value, and conjugated diene methods. The stability of MSO increased with increased proportions of HOSO, which was the source of 18:1n-9 in the blends. The ratio of 18:1n-9/18:2n-6 improved from 0.18 in MSO to 1.47 in the enzymatically interesterified blend. Calculated oxidizability and the results of oxidation tests of the blends confirmed the improvement in MSO stability by both physical blending and enzymatic interesterification.

JAOCS 75, 1155–1159 (1998).

KEY WORDS: Interesterification, lipase, melon seed oil, oxidizability, *sn*-2 position, triacylglycerols.

Melon seeds are a source of high-quality edible oil used in cooking and frying (1–3) at subsistence level in some African and Middle Eastern countries (3,4). Melon seeds are also used as thickeners in soup. The meal from melon seeds can be made into patties and served as meat substitute (5). Unfortunately, the stability of melon seed oil (MSO) is low. MSO is a liquid at room temperature, pale yellow in color, and has a mild odor. MSO has low acidity (2.7%, as oleic) and saponification number (202.4), and a high iodine value (123.9), reflecting its high degree of unsaturation (3). MSO has a high content of unsaturated fatty acids (79.5%) (6). Even though MSO is rich in essential fatty acid 18:2n-6 (64.5%) and may be an acceptable substitute for other highly unsaturated vegetable oils, such as corn oil, in the diet, MSO is susceptible to oxidation. The stability or resistance of MSO to oxidation may be improved by blending and/or interesterification with more stable tropical oils, such as palm oil and safou oil (7). Much research has been published on the oxidative stability of vegetable oils (8–12), but little has been reported on the stability of melon seed oil.

Interesterification of vegetable oil blends and hydrogenation (13) are becoming important processes for modification of the physical and functional properties of oils. For instance, blending of a high-oleic sunflower oil at different proportions with polyunsaturated vegetable oils has been used to prepare more stable edible oils (14). Blending or physical mixing of oils has some problems. Because of the molecular size differences, two oils may be incompatible with one another and may form eutectic mixtures (15). Hydrogenation has been a useful process to increase the stability of edible oils because it changes polyunsaturated fatty acids to monounsaturated and saturated fatty acids (9). However, concern about the nutritional safety of partially hydrogenated oils is increasing (16–19). To counter *trans* fatty acid problems that are associated with hydrogenation, interesterification may be used. Nowadays, the stability of oils can be improved through changes in triacylglycerol (TAG) composition and/or structure. This can be done through plant breeding or by enzyme-directed interesterification (20) with more stable fatty acids. Rearrangement of fatty acids of TAG molecules through interesterification alters the initial properties of oils and leads to the formation of new products. Lipase-catalyzed reactions, unlike chemical interesterifications, do not produce by-products; they give improved products with better quality (21). Lipase-catalyzed interesterification is currently used for the biotechnological processing of fats and oils (22). Enzymatic modification of the MSO fatty acid profile by incorporation of eicosapentaenoic acid, 20:5n-3 (23), and oleic acid, 18:1n-9 (6), has been explored.

This paper reports on the influence of lipase-catalyzed modification on the oxidative stability of MSO and the fatty acid distribution at the *sn*-2 position of TAG.

MATERIALS AND METHODS

Materials. Dried melon seeds (*Citrullus colocynthis* L.) of the Cucurbitaceae family, mainly imported from Nigeria, were purchased from Tropical Foods Market (Atlanta, GA). High-oleic sunflower oil (HOSO), commercially known as Trisun-80 oil, was supplied by SVO Enterprises (Eastlake, OH). Pancreatic lipase (E.C. 3.1.1.3, type II, from porcine pancreas)

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used for the *sn*-2 positional analysis of fatty acid was obtained from Sigma Chemical Co. (St. Louis, MO). A nonspecific *Pseudomonas* sp. lipase PS30 (36,600 U/g), used as biocatalyst for interesterification reactions of blends, was purchased from Amano Enzyme Co. Ltd. (Troy, VA). The 2-monoacylglycerol (2-MAG) was from Aldrich Chemical Company Inc. (Milwaukee, WI). All organic solvents were high-performance liquid chromatography grade.

Extraction. Melon seeds (100 g) were homogenized with a Waring blender (Dynamics Corporation of America, New Hartford, CT). The crude melon seed oil was extracted in a 500-mL flask with 400 mL of chloroform/methanol (2:1 vol/vol) in a Soxhlet apparatus for 8 h. Solvent was evaporated with a rotatory evaporator (Labconco Corporation, Kansas City, MO). For scale-up interesterification, MSO from several extractions were pooled to obtain enough oil as starting raw material.

Interesterification. Scaled-up interesterification of blends of MSO and HOSO was performed by adding 5 g of *Pseudomonas* sp. lipase PS30 (10%, w/w of reactants) and water (5%, w/w of enzyme) to the reactants, composed of a mixture of MSO and HOSO. The reactants, 50 g of each blend (i.e., 90:10; 80:20; 70:30 and 50:50, w/w proportions) were diluted with 200 mL hexane and incubated in a waterbath (New Brunswick Scientific Co. Inc., Edison, NJ) at 55°C at 200 revolutions/min for 36 h.

Sample purification. The enzyme was removed by passing the reaction product mixture through an anhydrous sodium sulfate column and a filter paper. The lipase-catalyzed interesterfication resulted in a mixture of products that contained TAG, diacylglycerols (DAG), MAG and free fatty acids (FFA). For this reason, interesterified blends were subjected to further purification prior to the oxidation tests. Among the different procedures for purification of oils, those involving distillation under high vacuum, as used here, are thought to be the most efficient (24).

For deacidification of the lipase-catalyzed blends, short-path distillation was used. Short-path distillation offers a gentle separation of heat-sensitive materials at low temperatures by combining the concept of distillation at low pressures with a wiped-film distribution of the feed for improved heat transfer, short residence time, and low product hold-up to achieve the best fractionation. After interesterification, interesterified blends were purified in a KDL-4 short-path distillation apparatus (UIC, Joliet, IL). The conditions used for distillation were: feed heat 20°C, evaporator heat 205°C, vacuum 0.04–1 mm Hg, condenser temperature −2.5°C, feed rate 400 mL/h, and a wiper speed of 400 rpm. The lipase-interesterified blends were distilled three times under the selected conditions, retaining the undistilled fraction each time. Volatiles collected from the distillation were removed. The feed and residue (TAG) were analyzed for fatty acid composition and acid value. The FFA content of feed was reduced by 20–25% after distillation. FFA were analyzed by the AOCS method (25).

Fatty acid profile and sn*-2 positional analysis*. The fatty acid composition of TAG of oils was determined as described previously (6). The distribution of fatty acids at the *sn*-2 position of TAG was determined by a modified method of Luddy *et al.* (26). TAG were isolated by thin-layer chromatography (TLC) and hydrolyzed with a *sn*-1,3-specific porcine pancreatic lipase, and the resulting 2-MAG were separated by TLC developed with hexane/ethyl ether/acetic acid (50:50:1, vol/vol/vol) prior to gas–liquid chromatography (GLC) (6).

Oxidation tests. Oil samples were evaluated for oxidative stability by the AOCS Oxidative Stability Instrument method (27) at 110°C. The Omnion Stability Instrument (Omnion, Inc., Rockland, MA) was equipped with an IBM-compatible computer and printer; two electric heating chambers, capable of heating 24 samples; two digital temperature controllers; gauge pressure; conductivity probes; and sample tubes. Peroxide values (PV) were determined at 60°C according to the AOCS method (28). Conjugated diene contents were determined with a Beckman DU-64 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) by measuring the absorbance at 234 nm of oil samples diluted in isooctane to a final concentration of 0.5 g/L (29). The oxidizability, a parameter used to predict susceptibility to oxidation, was calculated from the fatty acid composition according to Cosgrove's formula as modified by Neff *et al.* (12).

Statistical analysis. The analysis of variance with multiple comparisons was used to determine the significance of differences among groups and treatments, physical mixing and lipase-catalyzed interesterification (30). Significance level was $P < 0.05$.

RESULTS AND DISCUSSION

Fatty acid composition. Purified TAG of lipase-interesterified blends contained some FFA as observed on TLC. The FFA content of the physical blend was about 0.02%, whereas that of lipase-interesterified blends varied from 0.08 to 2.4%, as determined by the AOCS method (25). There was no significant difference between the fatty acid composition of samples before and after short-path distillation ($P > 0.05$). Table 1 shows the fatty acid composition of MSO, HOSO, and their blends. With an increase in HOSO proportion in the blends, the 18:1n-9 incorporation into or content in MSO increased while 18:2n-6 content decreased. This was observed for both physical blends and lipase-catalyzed interesterified blends. MSO had the highest oxidizability (0.661) and HOSO had the lowest (0.122). With increasing amounts of HOSO, the calculated oxidizability of MSO (0.661) decreased to 0.385 and 0.355 for the physical blend and the interesterified blend, respectively. The ratios of 18:1n-9/18:2n-6 were improved. Thus, in theory, the susceptibility of MSO to oxidation was greatly reduced by enzymatic interesterification and physical blending. The physical blends were significantly different from the interesterified blends for oxidizability. From this, the lipase-catalyzed interesterified blends would likely be more stable than the physical blends (12).

sn*-2 distribution.* To determine whether or not any notable change had occurred in the fatty acid distribution, an *sn*-2 positional analysis was performed on the TAG of MSO, HOSO,

TABLE 1
Fatty Acid Composition and Oxidizability of MSO, HOSO, Physical Blends, and Interesterified Blends

Sample	Fatty acid composition (mol%)[a]					
	16:0	18:0	18:1n-9	18:2n-6	18:1n-9/18:2n-6	Oxidizability[b]
MSO	13.3 ± 1.3[a]	8.3 ± 1.2[a]	12.5 ± 0.2[a]	65.9 ± 0.4[a]	0.18 ± 0.23[a]	0.661 ± 0.134[a]
Physical blends[c]						
9:1	12.7 ± 0.9[a]	8.0 ± 1.1[a]	18.7 ± 0.8[b]	60.8 ± 0.8[a]	0.30 ± 0.22[b]	0.611 ± 0.233[a]
8:2	11.0 ± 2.1[a]	7.5 ± 2.1[a]	25.8 ± 2.1[c]	55.7 ± 1.1[a]	0.46 ± 0.32[b]	0.562 ± 0.126[a]
7:3	10.6 ± 1.4[a]	7.4 ± 2.2[a]	33.4 ± 3.3[d]	48.6 ± 1.2[b]	0.68 ± 0.17[c]	0.492 ± 0.155[b]
1:1	9.6 ± 2.3[a]	6.4 ± 2.4[a]	46.4 ± 2.3[e]	37.6 ± 3.1[b]	1.23 ± 0.25[d]	0.385 ± 0.214[b]
Interesterified blends						
9:1	11.4 ± 0.7[a]	7.8 ± 2.3[a]	23.6 ± 3.2[b]	57.2 ± 3.3[a]	0.41 ± 0.19[b]	0.576 ± 0.133[a]
8:2	10.4 ± 0.2[a]	7.6 ± 3.3[a]	31.1 ± 2.1[c]	50.9 ± 1.2[a]	0.61 ± 0.27[e]	0.515 ± 0.222[a]
7:3	8.8 ± 1.1[b]	7.4 ± 2.5[a]	40.4 ± 1.3[d]	43.4 ± 0.7[b]	0.93 ± 0.42[f]	0.442 ± 0.134[b]
1:1	8.2 ± 2.1[b]	6.3 ± 1.2[a]	51.0 ± 0.1[e]	34.5 ± 0.5[b]	1.47 ± 0.36[g]	0.355 ± 0.112[b]
HOSO	5.8 ± 2.2[c]	3.9 ± 0.2[b]	79.6 ± 0.7[f]	10.7 ± 0.3[c]	7.43 ± 0.44[h]	0.122 ± 0.231[e]

[a]Mean of duplicate analysis. Means with the same letter in a column are not significantly different at $P < 0.05$.
[b]Oxidizability = [0.02(O%) + L% + 2(Ln%)]/100 [Cosgrove formula as modified by Neff *et al.* (12)].
[c]Blends are MSO:HOSO ratios. MSO, melon seed oil; HOSO, high-oleic sunflower oil; O, oleic; L, linoleic; Ln, linolenic.

and the physical and lipase-interesterified blends. Table 2 shows that, for MSO, HOSO and the physical blends, unsaturated fatty acids (i.e., 18:1n-9, and 18:2n-6) occupied the *sn*-2 position of TAG; this characteristic of highly unsaturated vegetable oils has frequently been observed by many researchers (31). Lipase-catalyzed interesterification resulted in a random distribution of fatty acids at the *sn*-2 position of TAG. The saturated fatty acids (16:0 and 18:0) of the interesterified blends were significantly greater at *sn*-2 than for the physical blends and could mean better absorption as 2-MAG. However, for unsaturated fatty acids (18:1n-9 and 18:2n-6), there were some differences, especially at the 7:3 and 1:1 ratios. It has been reported that a reduction of 18:2n-6 at the *sn*-2 position should improve the oxidative stability of the interesterified blends of highly unsaturated vegetable oils. The ratio 18:1n-9/18:2n-6 at the *sn*-2 position of TAG were in most cases significantly different between the physical and the interesterified blends, with higher values for enzymatically interesterified blends.

Oxidative stability index (OSI). OSI results of MSO, HOSO, and the blends are given in Table 3. The HOSO (10.70 h) was the most stable, and MSO (4.55 h) was the least stable, as predicted by the 18:1n-9/18:2n-6 ratio and oxidizability (Table 1). All blends were more stable than MSO. As the proportion of HOSO was increased, the stability of MSO also increased for both physical blends and lipase-interesterified blends. Analysis of variance for OSI values indicated that the physical blends were significantly different from the in-

TABLE 2
***sn*-2 Fatty Acid Composition of MSO, HOSO, Physical Blends, and Interesterified Blends**

Sample	Fatty acid composition (mol%) ± SD[a]				
	16:0	18:0	18:1n-9	18:2n-6	18:1n-9/18:2n-6
MSO					
Total lipid	13.3 ± 0.2[a]	8.3 ± 0.2[a]	12.5 ± 3.3[a]	65.9 ± 0.3[a]	0.18 ± 0.27[a]
sn-2 Position	ND	ND	24.3 ± 1.2[b]	75.7 ± 2.2[a]	0.31 ± 0.19[b]
Physical blends[b]					
9:1	0.6 ± 1.1[b]	0.2 ± 0.1[b]	27.0 ± 1.4[b]	72.2 ± 0.3[a]	0.37 ± 0.33[b]
8:2	0.7 ± 2.1[b]	0.4 ± 0.1[b]	30.7 ± 1.5[c]	68.2 ± 3.2[a]	0.45 ± 0.27[b]
7:3	0.9 ± 3.2[b]	0.7 ± 0.2[b]	34.6 ± 0.2[c]	63.8 ± 1.4[a]	0.54 ± 0.55[c]
1:1	1.3 ± 0.3[c]	0.7 ± 0.1[b]	44.9 ± 2.3[d]	53.1 ± 0.4[b]	0.84 ± 0.34[d]
Interesterified blends					
9:1	11.6 ± 0.2[d]	6.6 ± 0.9[c]	22.7 ± 1.3[b]	59.1 ± 3.3[a]	0.38 ± 0.37[b]
8:2	7.3 ± 1.4[e]	4.6 ± 0.7[c]	31.0 ± 0.5[c]	57.1 ± 1.2[b]	0.54 ± 0.27[c]
7:3	8.2 ± 0.3[e]	5.0 ± 0.2[c]	38.5 ± 0.8[d]	48.3 ± 1.1[b]	0.79 ± 0.24[d]
1:1	4.2 ± 2.1[f]	3.5 ± 0.8[c]	55.0 ± 2.3[e]	37.3 ± 1.3[c]	1.47 ± 0.23[e]
HOSO					
Total lipid	5.8 ± 1.3[f]	3.9 ± 1.2[d]	79.6 ± 0.2[f]	10.7 ± 0.1[d]	7.43 ± 0.21[f]
sn-2	ND	ND	82.8 ± 0.1[g]	17.2 ± 0.2[e]	5.17 ± 0.24[f]

[a]Mean ± standard deviation of duplicate analysis. Means with the same letter in a column are not significantly different at $P < 0.05$.
[b]Blends are MSO:HOSO ratios. ND, not detected. See Table 1 for other abbreviations.

TABLE 3
Induction Periods of MSO, HOSO, Physical Blends, and Interesterified Blends Determined with the Omnion Instrument[a]

Sample	OSI values ± SD at 110°C (h)[b]	OSI conversions to AOM values at 97.8°C (h)
MSO	4.55 ± 0.14[a]	10.52
Physical blends[c]		
9:1	8.80 ± 0.42[b]	20.80
8:2	8.87 ± 0.03[b]	21.00
7:3	9.05 ± 0.02[b]	21.44
1:1	9.97 ± 0.31[c]	23.64
Interesterified blends		
9:1	5.29 ± 0.04[a]	12.44
8:2	7.08 ± 0.04[d]	16.57
7:3	7.85 ± 0.07[d]	18.39
1:1	8.94 ± 0.05[b]	21.08
HOSO	10.70 ± 0.14[e]	25.40

[a]Omnion Stability Instrument (Omnion, Inc., Rockland, MA).
[b]Mean ± standard deviation of duplicate analysis. Means with the same letter in a column are not significantly different at $P < 0.05$.
[c]Blends are MSO:HOSO ratios. AOM, active oxygen method; OSI, oxidative stability index. See Table 1 for other abbreviations.

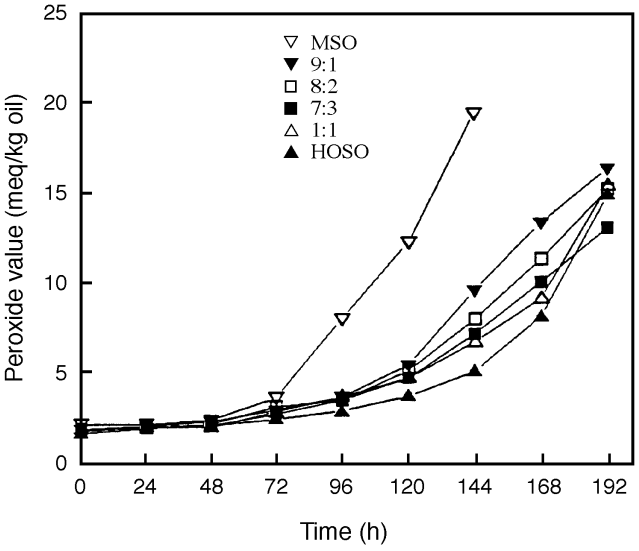

FIG. 2. Peroxide value (PV) of MSO, HOSO, and their interesterified blends as determined at 60°C. For abbreviations see Figure 1.

teresterified blends. Our results confirmed those reported by Neff *et al.* (14).

PV. Figures 1 and 2 show that the stability of blends at 60°C, based on PV, was significantly increased by blending and interesterification. The physical mixtures with a 5-d induction period were all significantly more stable than interesterified blends, but the interesterified blends were not significantly different from MSO after 3 d of oxidation.

Conjugated diene content. Figures 3 and 4 show that there were no significant differences observed in the slopes of the curves that represented accumulation of conjugated diene in the physical and interesterified blends.

From the results of oxidation tests, especially OSI and conjugated diene values, the stability of physical blends was not significantly different from that of the interesterified blends. Based on PV values, the physical blends were significantly more stable than the interesterified blends. The lower stability of the interesterified blends is due to the residual FFA (0.08–2.4%) content, which short-path distillation could not remove. Short-path distillation was not performed on the physical blends because it was not necessary. In addition to the fatty acid composition and fatty acid distribution at the *sn*-2 position of TAG, other factors, such as the structure of TAG, the removal of non-TAG components, such as tocopherols and phospholipids, during short-path distillation may have contributed to the low stability of interesterified blends.

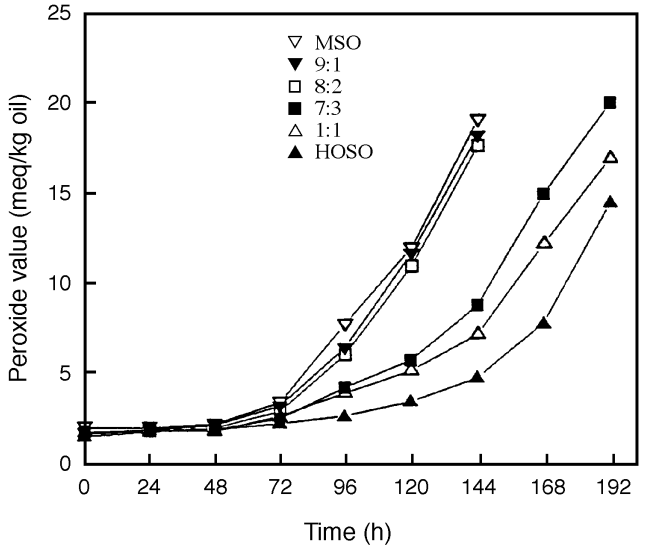

FIG. 1. Peroxide value (PV) of melonseed oil (MSO), high-oleic sunflower oil (HOSO), and their physical blends as determined at 60°C. Blends are MSO:HOSO ratios.

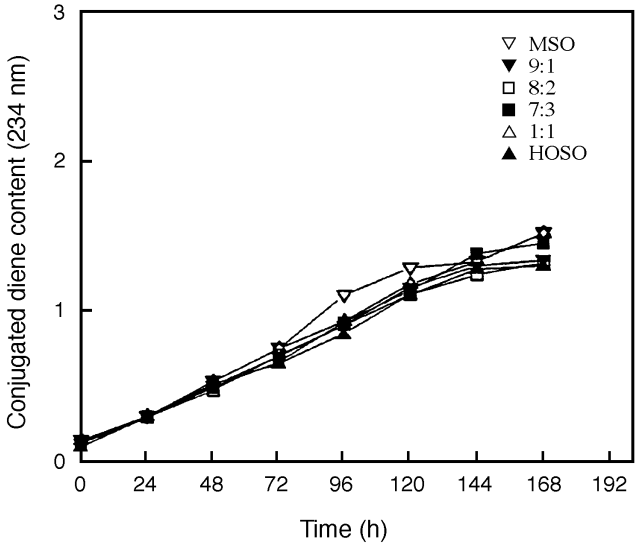

FIG. 3. Conjugated diene content (234 nm) of MSO, HOSO, and physical blends. For abbreviations see Figure 1.

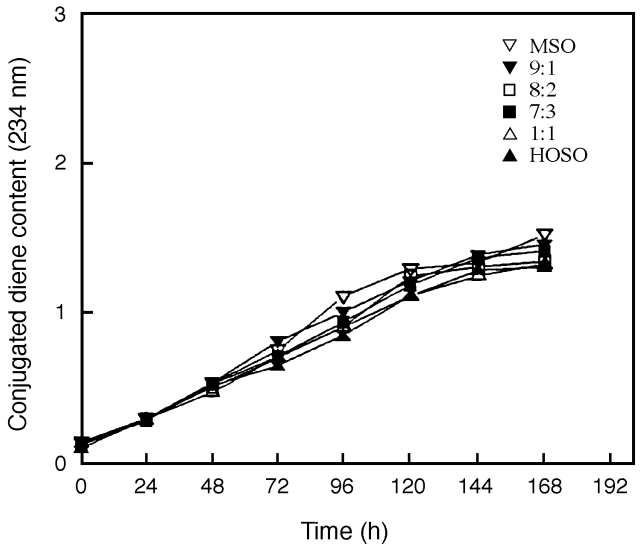

FIG. 4. Conjugated diene content (234 nm) of MSO, HOSO, and interesterified blends. For abbreviations see Figure 1.

Interesterification of MSO with HOSO remarkably changed the fatty acid composition of MSO. Also, a random removal of fatty acids at the *sn*-2 position occurred with the nonspecific lipase. However, a more stable MSO with acceptable levels of 18:2n-6 was obtained. Modification of MSO through interesterification with HOSO improved the stability of MSO.

ACKNOWLEDGMENTS

Contributed by the College Agricultural and Environmental Sciences, The University of Georgia. Research supported by Food Science Research.

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[Received December 1, 1997; accepted March 17, 1998]