Lipase-Catalyzed Incorporation of Oleic Acid into Melon Seed Oil

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ABSTRACT: The ability of lipase PS30 (*Pseudomonas* sp.) to modify the fatty acid profile of melon seed oil by incorporation of oleic acid (18:1n-9) was investigated. The transesterification was carried out in hexane in an orbital shaking water bath at 55°C for 24 h with methyl oleate (70% pure) as acyl donor. Oleic acid content increased from 13.5% to 53%, and linoleic acid (18:2n-6) content decreased from 65% to 33%. The incorporation of oleic acid into melon seed oil by *Pseudomonas* sp. lipase helped balance the fatty acid profile of the oil in terms of monounsaturated (18:1n-9) and essential fatty acids (18:2n-6). *JAOCS 74*, 177–179 (1997).

KEY WORDS: Acyl donor, essential fatty acid, fatty acid profile, lipase, melon seed oil, methyl oleate, transesterification, triacylglycerol.

The fatty acid profile of edible oils plays an important role in their stability and nutritional value (1). Monounsaturates (18:1n-9) and polyunsaturates (18:2n-6) have been found to be effective replacements for saturates as part of cholesterol-lowering diets. High levels of polyunsaturates decrease not only cholesterol and low-density lipoprotein (LDL) cholesterol levels but also the beneficial high-density lipoprotein (HDL) cholesterol levels (2). However, oils with substantial amounts of unsaturation, particularly 18:2n-6, are susceptible to oxidation and may produce products that contribute to atherosclerosis and carcinogenesis (3). Some studies with experimental animals indicate that excessive amounts of linoleic acid promote carcinogenesis (4).

Melon seed oil, rich in linoleic acid (64.5%), is used for frying and cooking in some African and Middle Eastern countries owing to its unique flavor (5). The modification of melon seed oil fatty acid composition by incorporation of eicosapentaenoic acid (20:5n-3) has been explored (6). Our objective for this study was to incorporate oleic acid (18:1n-9) into melon seed oil to help balance the fatty acid profile of melon seed oil in terms of the monounsaturate (18:1n-9) and essential fatty acids (18:2n-6). This modification may improve its oxidative stability and nutritional value.

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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). Methyl oleate (70% pure) was obtained from Aldrich Chemical Company Inc. (Milwaukee, WI). Silica gel 60 plates were from E. Merck (Darmstadt, Germany). Nonspecific lipase PS30 (*Pseudomonas* sp.) was purchased from Amano Enzyme Co. Ltd. (Troy, VA). All solvents were of high-performance liquid chromatography grade from J.T. Baker Inc. (Phillipsburg, NJ).

Extraction. Melon seeds (30 g) were homogenized with a Waring blender (Waring Products Division, New Hartford, CT). The melon seed crude oil was extracted with 200 mL of chloroform–methanol (2:1, vol/vol) in a Soxhlet apparatus for 8 h. Solvent was evaporated with a Büchi rotary evaporator (Postfach, Switzerland) and stored at –20°C until use.

Transesterification reaction. Enzymatic interesterification (ester exchange) was performed by continuously shaking, in a water bath at 55°C for 24 h at 200 revolutions/min, a mixture of melon seed oil and methyl oleate at 1:1 mole ratio in 3 mL hexane with 10% (w/w combined weight of substrates) nonspecific lipase (PS30) and 5% (w/w) water. Molecular sieves of 4Å were added after 2 h. All reactions were in duplicate.

Analysis of products. The enzyme was filtered through an anhydrous sodium sulfate column. Triacylglycerols (TAG) were isolated by preparative thin-layer chromatography (TLC) with petroleum ether/ethyl ether/acetic acid (90:10:1, vol/vol/vol) as developing solvent and detected with 0.2% 2,7-dichlorofluorescein in methanol. Fatty acid methyl esters (FAME) were obtained from TAG by methylating with 3 mL of 6% HCL in methanol solution at 70–80°C for 2 h and analyzed by gas–liquid chromatography (GLC) for fatty acid composition. A Hewlett Packard 5890 Series 11 gas chromatograph (Hewlett Packard, Avondale, PA) equipped with a DB-225 fused-silica capillary column (30 m × 0.25 mm i.d.; J&W Scientific, Folsom, CA) and flame-ionization detector (FID) was used to analyze samples. The injector and detector temperatures were 250 and 260°C, respectively. The column temperature was held at 205°C for 10 min and then programmed to 215°C at 20°C/min. Helium was the carrier gas, and the total flow rate was 23 mL/min. The relative content of fatty

acid methyl esters as mol% was quantitated by an on-line computer with heptadecanoic acid (17:0) as internal standard.

RESULTS AND DISCUSSION

The ability of lipase PS30 to incorporate oleic acid (18:n-9) into melon seed oil was investigated. Table 1 shows the fatty acid profile of unmodified and lipase-catalyzed modified crude melon seed oil. In crude unmodified oil, linoleic acid 18:2n-6 was the predominant fatty acid (65.4%), followed by palmitic acid 16:0 (14%), oleic acid 18:1n-9 (13.5%), and stearic acid 18:0 (9%). The fatty acid profile of melon seed oil total lipid obtained here was similar to that reported by Kamel *et al.* (7) where 18:2n-6 was 64.7%. Our results for 18:1n-9 resemble those reported by Akoh and Nwosu (5), and Huang *et al.* (6) but differ from those of Sawaya *et al.* (8) and Lazos (9).

Lipase-catalyzed interesterification of melon seed oil changed remarkably its fatty acid profile. Incorporation of 18:1n-9 resulted in a reduction of saturated fatty acids, 16:0 and 18:0 (Table 1). However, the decrease in 16:0 content was less than 50%, while 18:0 decreased by more than 50%. Oleic acid 18:1n-9 increased from 13.5% to about 53%, and linoleic acid 18:2n-6 decreased from 65.4% to 33 % with an increase in the mole ratio of methyl oleate to melon seed oil from 1 to 5 (Table 1). The original concentration of 18:2n-6 in melon seed oil before modification was 4.84 times that of 18:1n-9.

With an increase in the incubation time from 24 to 48 h, incorporation of oleic acid into melon seed oil increased only slightly, by 3.7% (Fig. 1). A higher incorporation rate of oleic acid was observed at an early stage of the reaction. Also, increasing the enzyme load increased the incorporation of 18:1n-9 (Fig. 2). An enzyme load of 5–10% is suggested to minimize the cost of the overall process. Above 10% lipase, incorporation of 18:1n-9 may result in a considerable removal of 18:2n-6. Therefore, a balance of 18:2n-6 and 18:1n-9 needs to be maintained for optimal nutritional benefits. Water is important for lipases to maintain their three-dimensional structure. We added trace amounts of water to the interesterification reactions (Fig. 3). Too much water resulted in poor incorporation of 18:1n-9 into melon seed oil TAG.

Because the 18:2n-6 content in crude oil approached that of other vegetable oils, such as sunflower, corn, and safflower, the producing countries may use melon seed oil as a replacement for these commercial vegetable oils in cooking and frying operations (5).

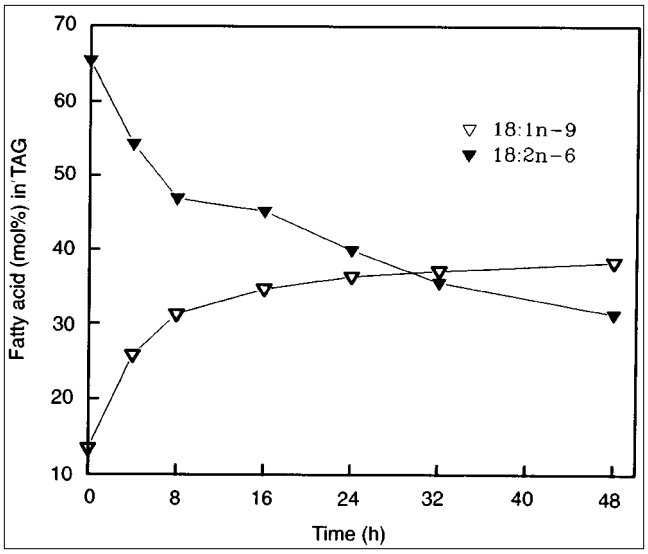

FIG. 1. Time course of incorporation of oleic acid (18:1n-9) into crude melon seed oil triacylglycerols (TAG) by *Pseudomonas* sp. lipase PS30-catalyzed transesterification in 3 mL hexane. Reaction mixture was incubated at 55°C in an orbital shaking water bath for 48 h at 200 rpm. Mole ratio of methyl oleate:melon seed oil was 1:1.

A panel of scientists from Iowa State University and of industrial consultants noted that raising oleic acid at the expense of linoleic acid would greatly increase the oxidative stability of soybean oil (10). The panel predicted that soybean oil that contains 40–60% oleic acid, 30–40% linoleic acid, with 2–3% of the remainder as saturated fatty acids, would have enough oxidative stability to eliminate the need for partial hydrogenation and would be low in saturated fats. Table 1 shows that we were able to achieve low saturation (13.7%), and a high monounsaturated fatty acid level (53.3%), and acceptable levels of 18:2n-6 (33%) with *Pseudomonas* sp. lipase PS30.

At a mole ratio of 1:2 (methyl oleate:melon seed oil), the amount of 18:1n-9 (43.2%) was almost equal to the amount of 18:2n-6 (40.2%). Together, 18:1n-9 and 18:2n-6 accounted for more than 80% of total fatty acids, which is similar to the

TABLE 1
Fatty Acid Profile (Mol%) of Crude and Lipase-Modified Melon Seed Oil[a]

Fatty acids	Unmodified crude melon seed oil	Modified melon seed oil (Methyl oleate/oil, mole ratio) 1	2	3	4	5
16:0	12.1	12.7	12.0	11.6	10.4	10.1
18:0	9.0	5.3	4.6	3.7	3.3	3.6
18:1n-9	13.5	34.8	43.2	48.9	52.1	53.4
18:2n-6	65.4	47.2	40.2	35.8	34.2	32.9
Total saturates	21.1	18.0	16.6	15.3	13.7	13.7
Total unsaturates	78.9	82.0	83.4	84.7	86.2	86.3
18:1n-9/18:2n-6	0.2	0.7	1.0	1.4	1.5	1.6

[a]Reaction mixtures in 3 mL hexane were incubated at 55°C in a orbital shaking water bath for 48 h at 200 revolutions/min.

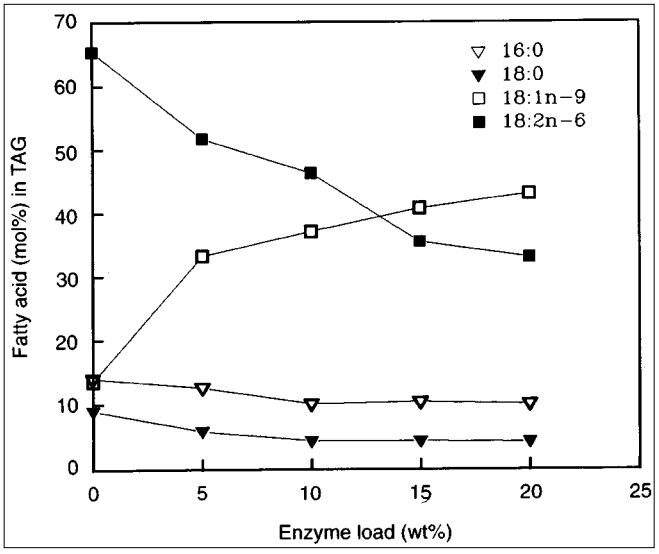

FIG. 2. Effect of lipase PS30 load on incorporation of oleic acid into melon seed oil. See Figure 1 legend for reaction condition.

amount of unsaturated fatty acids in Trisun 80 (SVD Enterprises, Eastlake, OH) oil, a high-oleic sunflower oil that contains 80% monounsaturates. Its characteristic high stability reflects the level of oleic acid. Nutritionally, it has been shown that the monounsaturate (18:1n-9) is capable of lowering total and LDL cholesterol while maintaining HDL levels (11). We found that the 18:1n-9/18:2n-6 ratio increased from 0.2 to 1.6 as the mole ratio of methyl oleate to melon seed oil was increased (Table 1). It is expected that this modified oil will be highly stable and may be nutritionally more beneficial than unmodified crude melon seed oil. Studies on the stability of the modified oil are in progress in our laboratory.

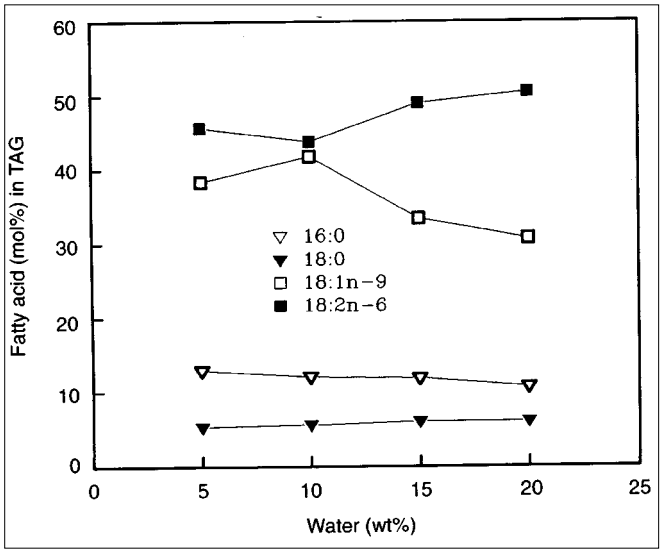

FIG. 3. Effect of added water on incorporation of oleic acid into melon seed oil with lipase PS30. The reaction condition was the same as in Figure 1.

High levels of polyunsaturated fatty acids (18:2n-6) decrease not only cholesterol and LDL levels but also the beneficial HDL level. When the level of monounsaturate (18:1n-9) is at least 50% or more of the total unsaturates and the level of saturates is low, a decrease in cholesterol and LDL level is observed, while a desirable level of HDL is maintained (12). Such beneficial ratios of 18:2n-6 and 18:1n-9 were obtained in the interesterified crude melon seed oil.

The results of this investigation demonstrate the ability of lipase PS30 *(Pseudomonas* sp.*)* to incorporate oleic acid into melon seed oil. Saturates, 16:0 and 18:0, were reduced. Modified melon seed oil TAG with balanced monounsaturate (18:1n-9) and essential fatty acids (18:2n-6) were produced. Work to improve melon seed oil oxidative stability by enzymatic modification is ongoing in our laboratory.

ACKNOWLEDGMENTS

Contributed by the College of Agricultural and Environmental Sciences, The University of Georgia. Research supported by Food Science Research No. 2526 GC 294000 and USDA HATCH Project No. GEO 00695.

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[Received April 23, 1996; accepted September 23, 1996]