Production of Cocoa Butter-like Fats by the Lipase-Catalyzed Interesterification of Palm Oil and Hydrogenated Soybean Oil

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ABSTRACT: Cocoa butter-like fats were prepared from refined, bleached, and deodorized palm oil (RBD-PO) and fully hydrogenated soybean oil (HSO) by enzymatic interesterification at various weight ratios of substrates. The cocoa butter-like fats were isolated from the crude interesterification mixture by fractional crystallization from acetone. Analysis of these fat products by RP-HPLC in combination with ELSD or MS detection showed that their TAG distributions were similar to that of cocoa butter but that they also contained MAG and DAG, which were removed by silica chromatography. The optimal weight ratio of RBD-PO to HSO found to produce a fat product containing the major TAG component of cocoa butter, namely, 1(3)-palmitoyl-3(1)-stearoyl-2-monoolein (POS), was 1.6:1. The m.p. of this purified product as determined by DSC was comparable to the m.p. of cocoa butter, and its yield was 45% based on the weight of the original substrates.

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KEY WORDS: Cocoa butter-type fats, interesterification, lipase, palm oil, soy oil.

World production of cocoa butter was about 675,000 metric tons in 2001 (1). Cocoa butter is currently the fat of choice in chocolate and other confectionery industries based on its important organoleptic and physical properties in these applications. Numerous factors, however, including the degree of uncertainty in supply, variability in quality, and price premium compared to other fats, have driven the search for alternatives. Numerous reformulated vegetable oils are currently produced as cocoa butter equivalents (CBE), with the potential market for CBE being as much as 10% of the total cocoa butter market (2,3).

Attempts have been made to prepare cocoa butter-like fats by interesterification of hydrogenated cottonseed oil and olive oil and subsequent fractionation (4). Edible beef tallow also has been solvent-fractionated from acetone to produce cocoa butter-like fractions (5). The preparation of cocoa butter substitutes by means of lipase-catalyzed intersterification has attracted recent attention (6–10) owing to the availability of lipases that catalyze the regioselective interchange of acyl groups at the 1- and 3-positions of the TAG structure, and such substitutes have been approved for food use (2).

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Numerous fats suitable for total or partial replacement of cocoa butter in confectionery products have been identified. Palm oil is an important fat used in the production of cocoa butter-like fats (7,8,11,12). There are few papers, however, regarding the production of cocoa butter-like fats from palm oils based on their geographical origin, which may affect the content of important TAG such as palmitoyl-oleoyl-stearoyl glycerol(POS) (11) in the final product. This paper reports the production of cocoa butter-like fats by lipase-catalyzed interesterification of palm oil and fully hydrogenated soybean oil (HSO) and compares the fatty acyl and TAG composition and melting profile of these products to cocoa butter.

MATERIALS AND METHODS

Materials. HSO (16% C16:0, 84% C18:0, iodine value <5) was a gift from Nabisco Brands (Indianapolis, IN). Nigerian palm oil was refined, bleached, and deodorized (RBD-PO) at the Food Protein Research and Development Center (College Station, TX). Lipozyme IM (an immobilized preparation of a 1,3-positionally specific lipase from *Rhizomucor miehei*) was a product of Novozyme (Franklinton, NC). TAG standards were purchased from Sigma (St. Louis, MO), and the cocoa butter standard was a gift from Loders Croklaan (Channahon, IL).

Methods. (i) Enzymatic interesterification. RBD-PO and HSO were blended at weight percent ratios of 3:1; 1.6:1; 1:1; 1:1.6; and 1:3. Interesterification of the blends was carried out in screw-capped sealed vials fitted with a magnetic stirring bar. The vials were placed into a water-jacketed beaker through which thermostatted water was circulated. Reactions were carried out at 70°C for 4 h with magnetic stirring at 200 rpm. The reaction mixtures (3 g, total substrate mixture) were heated for 5 min at 70°C before addition of the lipase (10 wt% of substrates). The optimal weight ratio of substrates (blend) was ascertained by determining the production of the major TAG component of cocoa butter, namely, POS.

(ii) Isolation of cocoa butter-like fat. Acetone fractionation of the interesterified blends was performed using a modification of the procedures reported previously (4,5). Acetone (7.5 mL/g of substrate) was added to the fat mixture at the end of the interesterification reaction. The warm acetone solution was quickly filtered through filter paper to remove the enzyme, the filtrate was cooled to room temperature (≈22°C), and any precipitated solids were removed by filtration. The filtrate was then cooled to 4°C for 4 h, and the precipitated

TAG crystals were filtered at 4°C to give the cocoa butter-like fat. The crystallized products were then subjected to GLC, RP-HPLC, HPLC-MS, and DSC analyses.

(iii) Purification of cocoa butter-like fats. Silica gel 60 (17.5 g, 40–63 μm particle size; Macherey-Nagel Polygosil 60-4063; Düren, Germany) was packed into a chromatography column (4 cm o.d. × 120 cm) that contained a glass wool plug covered with sea sand. A mixture of hexane/ether (200 mL of 85:15, vol/vol) was passed through the column at a flow rate of 2 mL/min under a slight nitrogen head pressure (void volume of the column was approximately 90 mL). The 4°C crystallized fat sample (1.27 g) dissolved in hexane/ether (8 mL, 85:15) was then applied to the head of the column. External heat with a heating tape was used at the top of the column to maintain dissolution of the solid TAG. The column was eluted with hexane/ether (85:15, 600 mL) at a flow rate of about 2 mL/min, fractions were collected (20 × 15 mL and 10 × 30 mL), and the solvent was removed with a rotary evaporator. Each fraction was reconstituted with ether (1 mL) and analyzed on a silica TLC plate (10 × 20 cm, 250 μm). Plates were developed with a solvent system composed of hexane/ether/acetic acid (80:20:1, by vol). After air-drying, the plates were sprayed with methanolic sulfuric acid (10%) and heated for visualization of lipid classes. Fractions containing only TAG were combined, and solvent was removed under a stream of nitrogen to give the purified fat product. The purified blends ($B_{1–5}$) obtained with the various mole ratios of reactants used are listed in Table 1.

(iv) GC analyses. The fatty acyl composition of the RBD-PO, HSO, and fat products (interesterified blends) was determined by converting their TAG into FAME (13). The FAME were analyzed by GLC on a Hewlett-Packard (Avondale, PA) model 5895 chromatograph equipped with a split capillary injector, an FID, and a Hewlett-Packard (model 3396) integrator. Separations were obtained on a 0.25 mm i.d. × 30 m SP2340 column from Supelco Inc. (Bellefonte, PA). The carrier gas was helium with linear velocity of 22.9 cm s^{-1} at 80:1 split ratio. Elution of FAME was carried out with temperature programming from 140 to 155°C at 0.5°C min^{-1}, then 155 to 200°C at 20°C min^{-1}. FAME were identified by comparison with standards, mixture M-100, obtained from Nu-Chek-Prep (Elysian, MN).

(v) RP-HPLC analysis. HPLC was carried out with a Hewlett-Packard model 1050 high-performance liquid chromatograph equipped with an autosampler, quaternary pump module, and a Varex (Burtonville, MD) ELSD II mass detector. The detector output was routed to a Hewlett-Packard Vectra XM24/100 MHz computer. The TAG mixtures were separated by nonaqueous RP-HPLC on a Beckman/Altex (Rainin, Woburn, MA) Ultrasphere ODS (5 μm) column (250 × 4.6 mm). Separations were carried out with acetone (solvent A) and acetonitrile (solvent B) as eluents at a flow rate of 0.8 mL/min and the following solvent gradient profile: initial condition 70:30 (volume percent A/B), hold for 5 min, to 100% A over 15 min, hold for 8 min, return to original conditions over 5 min, then hold for 5 min (total run time was 33 min). Estimation of total TAG equivalent carbon numbers (13) for separated peaks was made by comparison with standard TAG mixture G-1 (Nu-Chek-Prep). Characterization of TAG molecular species (14) was carried out on a Hewlett-Packard Model 5989 LC-MS with 59987A electrospray LC-MS interface operated in the atmospheric pressure chemical ionization mode with the following parameters: N_2 drying gas, 350°C, 13 mL; N_2 nebulizing gas, 350°C, 50 psi; quadrapole temperature 150°C, ionizing voltage 100 V; HPLC conditions used in LC-MS were as described earlier.

(vi) DSC analysis. Melting profiles for the TAG products were obtained with a PerkinElmer Pyris 1 differential scanning calorimeter (PerkinElmer Corporation, Norwalk, CT). The samples (5–10 mg) were accurately weighed into aluminum pans, which were then hermetically sealed with a "cold welder." An empty pan was used as the reference. The samples were held for 5 min at 0°C and thereafter subjected to a heating rate of 5°C/min from 0 to 50°C. The data were collected by a PerkinElmer data station, model 3000, and analyzed by a standard DSC program. All samples, including the cocoa butter, were tempered at 15°C for 48 h prior to DSC analysis.

TABLE 1
Fatty Acyl Composition of Cocoa Butter (CB), Refined-Bleached-Deodorized Palm Oil (RBD-PO), Hydrogenated Soy Oil (HSO), and Interesterified RBD-PO/HSO Blends[a]

	FA (%)[b,c]				
	C14:0	C16:0	C18:0	C18:1	C18:2
PO	1.6	39.9	7.3	45.8	5.4
HSO	—	16.1	83.9	—	—
CB	0.7	25.2	35.5	35.2	3.2[a]
B_1 (3:1)	0.9[a]	31.7[a]	32.2[a]	18.6[a]	1.6[b]
B_2 (1.6:1)	0.9[a]	32.2[b]	38.5[b]	23.8[b]	2.3[c]
B_3 (1:1)	0.7[b]	28.2[c]	48.8[c]	20.4[c]	2.2[c]
B_4 (1:1.6)	0.6[b]	25.5[d]	58.8[d]	13.8[d]	1.3[d]
B_5 (1:3)	0.4[c]	19.5[e]	70.1[e]	6.7[e]	—

[a]Blends ($B_{1–5}$) were obtained by enzymatic interesterification of RBD-PO and HSO at the weight percent ratio indicated (RBD-PO:HSO) after purification.
[b]Fatty acyl composition of RBD-PO, HSO, and the purified fat blends as determined by GC after conversion to FAME.
[c]Values in the same column with different roman superscripts are significantly different at $P < 0.05$; each value is the mean (±0.20%) of two different experiments.

RESULTS AND DISCUSSION

The weight percent distribution of fatty acyl groups in the starting RBD-PO, HSO, their interesterified blends, and cocoa butter are listed in Table 1. Stearic acid (C18:0) was the main FA in HSO, and with increasing amounts of HSO in the interesterification mixture, more stearic acid was incorporated into the interesterified blends. This is in agreement with the observation of Mohammed *et al.* (15), where an increase in the percentage of HSO in the blends of the lipase-catalyzed interesterification of cottonseed oil and HSO led to an increased amount of stearic acid incorporation into the blends. Oleic acid (C18:1) and palmitic acid (C16:0) were the dominant FA of

the RBD-PO of Nigerian origin and hence were the second-most dominant FA present in the interesterified blends.

Cocoa butter is composed predominantly of the FA palmitic, stearic, and oleic (Table 1), which typically account for 95% of the total FA present (16). The purified interesterified blends (B_{1-5}) prepared in this study also contained these FA as their main FA constituents (Table 1). Blend B_2 was a semisolid or plastic solid with a FA composition of about one-fourth unsaturated acids, mainly oleic, which gave it a similarity to cocoa butter although the palmitic/stearic ratio was higher.

POS is the major TAG component of cocoa butter (Fig. 1A). The main TAG component of palm oil is POP, which is converted to POS and SOS during the interesterification with HSO. As it is the fatty acyl groups in positions 1 and 3 of the palm oil TAG that are exchanged with stearic acid when using a 1,3-specific lipase, an interesterification product with a TAG distribution more closely resembling cocoa butter was obtained, unlike chemical interesterification where stearic acid would be randomly distributed on the glycerol backbone.

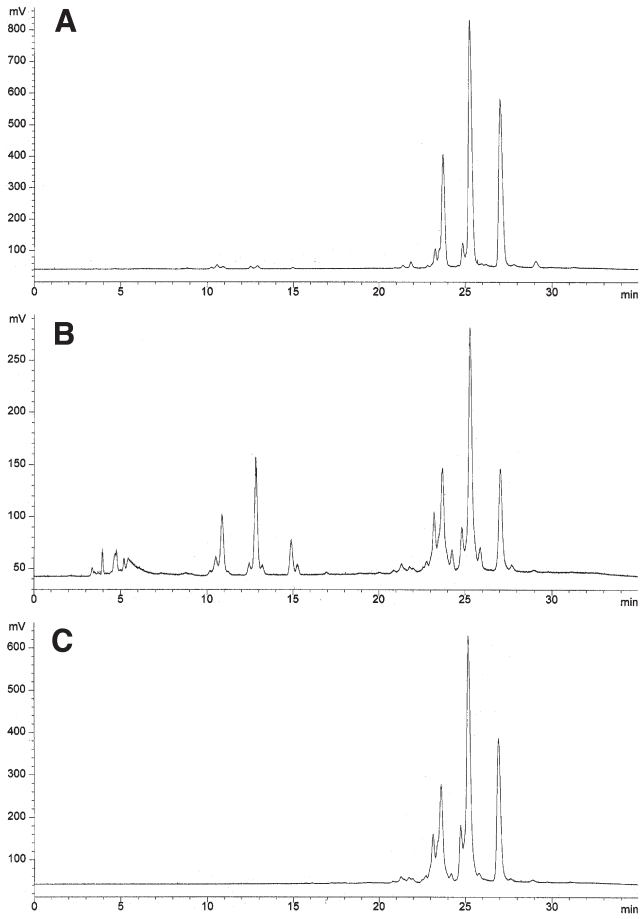

FIG. 1. RP-HPLC chromatograms showing the separation of TAG for (A) cocoa butter [retention time (min), TAG]: 23.5, POP; 25.0, POS; 26.7, SOS; (B) chromatogram of crude interesterified blend B_2: 23.6, POP; 25.2, POS; 27.0, SOS; and (C) chromatogram of purified interesterified blend B_2: 23.6, POP; 25.1, POS; 27.0, SOS. Abbreviations: P = palmitoyl, O = oleoyl, S = stearoyl.

With the exception of blend B_5, POS was the major TAG in the blends, with blend B_2 having the highest amount of POS (Table 2). In blend B_5 the major TAG was SOS, which is a result of the high mole ratio of HSO used to prepare this blend. Comparison of the RP-HPLC chromatogram of the blends to that of cocoa butter showed that blend B_2 (Fig. 1B) had a TAG distribution most similar to that of pure cocoa butter, but it contained significant amounts of MAG and DAG (~5 to 8%). The identity of the major TAG peaks was confirmed by HPLC combined with MS detection. Because blend B_2 contained the largest POS content, it was regarded as the optimal blend (weight ratio) among the others and was therefore further purified by silica column chromatography to remove the contaminating partial glycerides (Fig. 1C). The yield of isolated cocoa butter-like fat was 45.6% based on the weight of the original substrates. Other authors have achieved different yields of CBE. Chang *et al.* (4) reported a yield of cocoa butter-like fat of 19% when fully hydrogenated cottonseed oil (HCO) was interesterified with olive oil at a weight ratio of 1:1 and up to 53.0% when CBE were synthesized from palm oil and HCO in supercritical CO_2 (9).

Our purified B_2 blend product had a m.p. of 33.8°C compared to a pure cocoa butter with a m.p. of 31.3°C as measured by DSC (Figs. 2A and 2B, respectively). The m.p. of 33.8°C observed for blend B_2 compares well to the m.p. of cocoa butter as reported by other authors: 36°C for Hershey cocoa butter, Chang *et al.* (4); 33°C, 32°C, Jensen (17); and 31°C, Smith (18). It also compares well to cocoa butter-like fats produced by other authors for CBE (34.3°C) synthesized from palm oil (9), and for a cocoa butter-like fat (39°C) produced by interesterification of HCO and olive oil at 1:1 substrate weight ratio (4). The DSC melting profile for blend B_2, although shifted to slightly higher temperatures, also compares fairly well with the melting profile of cocoa butter. Since melting is characterized by the large endothermic deflection of the curves, the similarity in melting of the two materials

TABLE 2
Distribution of TAG in the Structured Lipid Products Obtained from the Lipase-Catalyzed Interesterification of Palm Oil (RBD-PO) and Hydrogenated Soy Oil (HSO)

		TAG[c,d] (wt%)		
Sample[a]	RBD-PO/HSO Ratio[b]	POP	POS	SOS
B_1	3:1	14[a]	33[a]	11[a]
B_2	1.6:1	11[a]	39[b]	23[b]
B_3	1:1	6[b]	35[a]	31[c]
B_4	1:1.6	3[c]	30[c]	37[d]
B_5	1:3	1[c]	16[d]	42[e]
Cocoa butter	—	17	45	31

[a]Interesterified blend obtained after purification of the crude product mixture by acetone crystallization and silica column chromatography.
[b]Weight percent ratio of RBD-PO to HSO used for interesterification.
[c]Major TAG present in blends and cocoa butter. TAG (wt%) distribution of HSO (PPS, 4; PSS, 43; and SSS, 49) and RBD-PO (SOP, 6; OPP, 36; OOP, 26; LPP, 9; and POL, 9). P = palmitoyl; S = stearoyl; O = oleoyl; L = linoleoyl residues on the glycerol backbone.
[d]Values in the same column with different lowercase roman superscripts are significantly different at $P \leq 0.10$; each value is the mean (±2.0%) of two different experiments.

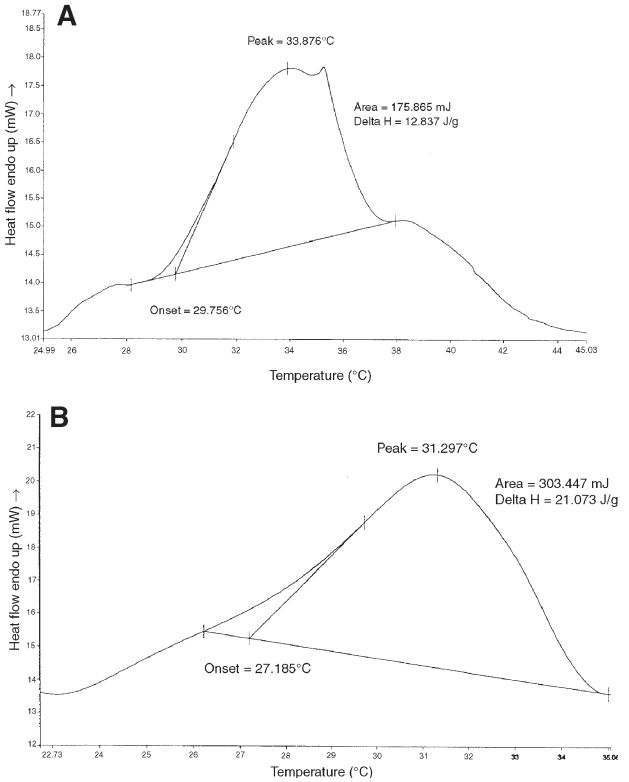

FIG. 2. DSC chromatograms for purified interesterified palm oil and hydrogenated soy oil blend B_2 (A) and cocoa butter (B).

is evident in that both melt rather sharply and completely at body temperature. This is one of the properties of cocoa butter that makes it desirable as a confectionery fat (2). Fraction B_2 resembles cocoa butter in its glyceride composition, in that it is composed primarily of 2-oleoyl disaturated TG of palmitic and stearic acids, but its higher stearic acid content is reflected in its higher m.p. compared to cocoa butter. Another important characteristic of cocoa butter is the steepness of the melting profile (solid fat content as a function of temperature) over a narrow temperature range (27 to 33°C). The melting profile for blend B_2 was similar to that of cocoa butter in that it melts over the temperature range of 27–36°C. For some applications in coating or confectionary fats, the higher-melting characteristics of blend B_2 may be advantageous. By incremental blending of other fractions (B_4 and B_5, Table 1), that contain higher proportions of SOS TAG, with blend B_2, the melting range of this semisolid fraction can be raised effectively, which may further expand the utility of this plastic fat in coating applications.

Based on the data presented here, with 10 wt% of enzyme total fat weight, a 1.6:1 ratio of palm oil to HSO is required for the best yield of cocoa butter-like fats. This and previous studies (7,10) on the production of cocoa butter-like fats by lipase-catalyzed interesterification of vegetable oils, however, show that the CBE-type products obtained contain significant amounts of DAG, which makes these products unsuitable as CBE. In this study, we have shown that purification of the products eliminates the DAG, thus leading to a product whose TAG composition more closely matches that of cocoa butter as shown by RP-HPLC and the HPLC-MS analysis of the TAG.

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