# Fractionation Procedures for Obtaining Cocoa Butter-Like Fat from Enzymatically Interesterified Palm Olein

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**Solvent-free lipase-catalyzed incorporation of stearic acid in palm olein by the 1,3-regiospecific Novo Lipase Lipozyme IM20 resulted in the formation of a complex mixture of fatty acid glycerides and free fatty acids. The stearoyl incorporation in palm olein gave rise to the formation of 39.3% of the desired cocoa butter-like triglycerides in the fatty acid glyceride portion, namely distearoyl-oleoyl-glycerol (SOS), palmitoyl-oleoyl-stearoyl-glycerol (POS) and dipalmitoyl-oleoyl-glycerol (POP). A combination of fractionation steps involving initially the removal of free fatty acids (FFA) from the product mixture by steam distillation under vacuum, followed by fractional crystallization of the fatty acid-free glycerides in hexane and/or acetone, gave a fat, whose triglyceride composition and melting profile were comparable to that of cocoa butter as adduced by reversed-phase high performance liquid chromatography (HPLC) and differential scanning calorimetry (DSC). The yield of the cocoa butter-like fat was approximately 25% of the weight of the original palm olein.**

KEY WORDS: Cocoa butter-like fat, fractional crystallization, interesterification, lipase, palm olein, steam fractionation, stearic acid.

Cocoa butter is an important and expensive raw material used in the chocolate and related confectionery industries. It contains substantial quantities of 2-oleoyl glycerides of palmitic and stearic acid, which confer the valuable crystallization and melting characteristics so essential in providing, in chocolate confectionery, a sharp melting in the region of body temperature (1,2). Furthermore, there are a number of fats suitable for total or partial replacement of cocoa butter components in these confectionery products. Palm oil has been identified as an important source oil in the development of such cocoa butter substitutes (2–6). The lipase-catalyzed incorporation of stearic acid in palm oil, palm stearin and palm olein by the 1,3-regiospecific Novo Lipase Lipozyme 1M20 (Copenhagen, Denmark) resulted in the formation of a complex mixture of triglycerides which contained 40–50% of cocoa butter-like triglycerides, namely distearoyl-oleoyl-glycerol (SOS), palmitoyl-oleoyl-stearoyl-glycerol (POS) and dipalmitoyl-oleoyl-glycerol (POP) (7).

Enzymatic interesterification of the palm oil substrates with stearic acid resulted in the release of an equivalent amount of free fatty acids (FFA). Thus, appropriate fractionation procedures will have to be devised to separate the residual and released free fatty acids from the glycerides, and the latter into the desired cocoa butter-like components. Steam fractionation under vacuum is a distillation process that could lead to the complete physical separation of lower boiling fractions, such as free fatty acids, from higher boiling components, such as oils and fats. The fatty acid-free glyceride fraction can then be subjected to conventional fat fractionation techniques such as countercurrent liquid-liquid extraction and crystallization from solvents to produce fats with distinct melting profiles (8–10). Pszczola (11) describes a wet-fractionation plant for the production of specialty fats and high-stability oils where the process involves cooling the oil in a solvent to a specific temperature, so that part of the oil solidifies and the solids can be filtered out of the oil. The process is repeated at lower temperatures so that different crystals of the solid fat can be solidified and filtered out. In general, the process involves refrigeration, solvent fractionation, evaporation and solvent recovery.

This work describes the various fractionation steps based on the above fractionation concepts for obtaining the desired cocoa butter-like fat from interesterified palm olein and the characterization of the various fat and oil fractions in terms of their diglyceride and triglyceride compositions and of their melting profiles as determined by reversed-phase high performance liquid chromatography (HPLC) and differential scanning calorimetry (DSC), respectively.

## EXPERIMENTAL PROCEDURES

*Materials.* Refined, bleached and deodorized (RBD) palm olein was obtained from a local palm oil refinery (Lam Soon, Petaling Jaya, Malaysia). Palm olein and palm stearin are the liquid and solid fractions of palm oil obtained by progressive cooling of palm oil under fully controlled conditions. Prime processed cocoa butter from select Malaysian cocoa beans was obtained from a local cocoa butter producer (Upali, Shah Alam, Malaysia).

Industrial-grade stearic acid (98.3%) derived from palm oil was supplied by a local oleochemical plant (Southern Acids, Klang, Malaysia). The following reference diglycerides, triglycerides and fatty acids (99% purity) were obtained from Sigma Chemical Company (St. Louis, MO) and Larodan (Malmo, Sweden): 1,2-dipalmitin; 1,2-distearin; 1,2-diolein; 1,3-dipalmitin; 1,3-distearin; 1,3-diolein; OOO; POO; POP; PPP; SOO; PPS; SOS; SPS; SSS; palmitic acid and linoleic acid. As standards for OOL, POL, PPL and POS were not available commercially, peak identification was based on synthesized triglycerides (OOL, POL, PPL) and on the POS component of natural cocoa butter. Based on the 1,3-regiospecificity of the Lipozyme-catalyzed interesterification of triolein with palmitic and linoleic acids, six chromatographically detectable triglycerides were identified as OOO, POO, POP, POL, LOL and OOL, based on their degree of unsaturation and molecular weight (11–13). Similarly, enzymatic interesterification of tripalmitin with linoleic acid gave PPP, PPL and LPL. All other chemicals and solvents used were obtained commercially and were of the highest purity available.

The 1,3-specific Novo lipase Lipozyme IM20 (activity 28.1 BIU/g) was a preparation of *Mucor miehei* lipase

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(EC 3.1.1.3) immobilized on a macroporous anion exchange resin (donated by Novo Nordisk A/S, Kuala Lumpur, Malaysia). It is optimally used at temperatures between 60°C and 70°C.

*Enzymatic interesterification.* Solvent-free enzymatic interesterification of palm olein (1 kg) with stearic acid (0.5 kg) was carried out in a closed 5-L glass reactor equipped with a mechanical stirrer (200 rpm) and heater (60°C) for 20 hr in the presence of Lipozyme (10% w/w oil) and water (1% v/w oil). The product melt was decanted after sufficient time was allowed for the immobilized enzyme to settle.

*Fat fractionation.* The interesterified palm olein was subjected to steam distillation under vacuum (6 mm Hg) at 260°C for about 1.5 hr to give a steam-distilled fatty acid distillate (40.3%; 91.90% FFA) and the residual solid glyceride fraction (59.7%; 0.04% FFA). Solvent fractionation of the steam-fractionated glyceride fraction was carried out from either hexane and/or acetone. Three solvent-fractionation procedures were adopted:

Procedure A: A 1:10 (w/v) glyceride-hexane solution was left at 4°C for 24 hr. The precipitated fat F1(A) was filtered off, and the filtrate was evaporated to dryness. The mother liquor was then dissolved in acetone (1:10 w/v) and cooled at 4°C/24 hr. A second precipitated fat fraction F2(A) was obtained. The solvent was evaporated from the mother liquor to yield an oil fraction F3(A).

Procedure B: The above solvent fractionation procedure was repeated but instead with 1:5 (w/v) glyceride-hexane and 1:5 (w/v) mother liquor-acetone crystallization ratios to give the respective fractions F1(B), F2(B) and F3(B).

Procedure C: A 1:10 (w/v) glyceride-acetone solution was left at room temperature (*ca.* 25°C) for 24 hr to give a fat fraction F1(C), which was filtered off. The filtrate was then cooled at 4°C/24 hr to give another fat fraction F2(C). The final filtrate was evaporated to dryness to give an oil fraction F3(C).

*Analysis of glycerides.* The glyceride compositions of the various fractionated fats and oils were obtained by HPLC in a Waters HPLC System equipped with an RI 410 refractometer detector (Milford, MA). A Supelco LC 18 (25 cm × 4 mm i.d.) column (Bellefonte, PA) was used for the analysis with a mobile phase of 63.6:36.4 (v/v) acetone and acetonitrile at a flow rate of 1 mL/min. Each sample was dissolved in tetrahydrofuran to make a 5% solution. The injection volume from an auto-injector was 20 μL per injection. Both detector and column were maintained at 35°C. The melting characteristics of the various fat fractions were analyzed by a Mettler TA 4000 differential scanning calorimeter (Greifensee, Switzerland). The samples (*ca.* 5 mg) were subjected to a heating rate of 10°C/min from 10°C to 60°C and from 30°C to 90°C for the lower and higher melting fat fractions, respectively.

## RESULTS AND DISCUSSION

*Composition of glyceride fractions.* The total product mixture of the enzymatic interesterification reaction was a solid at room temperature which, upon steam distillation under vacuum, gave a fatty acid distillate and the residual solid interesterified palm olein (IPO). Table 1 shows a comparison (by HPLC) of the diglyceride and triglyceride compositions of the IPO and the three major fractions obtained therefrom by hexane and/or acetone fractionation. The IPO contained 10.2% diglycerides, and the rest was made up of thirteen chromatographically detectable triglycerides. The stearoyl incorporation had resulted in the formation of about 39.3% of the cocoa butter-like triglycerides, the disaturated-monounsaturated fatty acid glycerides (S2U), namely distearoyl-oleoyl-glycerol (SOS), palmitoyl-oleoyl-stearoyl-glycerol (POS) and dipalmitoyl-oleoyl-glycerol (POP). The distribution of the diglycerides and types of triglycerides (S3, S2U, SU2 and U3) within the three fractions, and their respective fractional yields, are as indicated in Table 2. The S2U-rich fractions contained between 51.7 to 67.7% of the cocoa butter-like triglycerides (SOS/POS/POP) present in the original IPO. Of particular interest is the F2(A) fraction, which

TABLE 1

**Composition of Various Fractions (F1,F2,F3) of Interesterified Palm Olein Obtained by Different Solvent Fractionation Procedures (A,B,C)**

| Fraction | Diglycerides (%) | Triglycerides[a] (%) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | OOL | POL | PPL | OOO | POO | POP | PPP | SOO | POS | PPS | SOS | SPS | SSS |
| Interesterified palm olein | 10.2 | 1.6 | 4.7 | 1.9 | 2.1 | 12.2 | 9.9 | 1.8 | 9.5 | 18.6 | 5.8 | 10.8 | 7.4 | 3.5 |
| F1(A)[b] | 16.4 | 0.3 | 0.3 | 0.4 | — | 1.3 | 3.5 | 5.8 | 1.0 | 9.1 | 20.1 | 6.8 | 24.2 | 10.8 |
| F2(A)[b] | 0.9 | 0.4 | 1.4 | 0.1 | — | 2.3 | 15.3 | 1.0 | 2.9 | 44.1 | — | 29.6 | 0.5 | 1.5 |
| F3(A)[b] | 14.3 | 0.8 | 9.8 | 3.4 | 4.8 | 25.9 | 9.7 | — | 17.7 | 8.7 | — | 1.9 | — | — |
| F1(B) | 13.1 | — | 0.3 | 0.4 | — | 0.7 | 2.9 | 5.4 | 0.6 | 7.7 | 20.3 | 6.8 | 28.8 | 13.0 |
| F2(B) | 3.3 | 0.7 | 2.1 | 2.5 | 0.8 | 6.0 | 16.0 | — | 6.1 | 38.7 | — | 23.1 | — | 0.7 |
| F3(B) | 15.6 | 4.1 | 10.4 | 3.4 | 5.2 | 26.8 | 7.8 | — | 19.0 | 6.2 | — | 1.5 | — | — |
| F1(C) | 2.7 | 0.4 | 1.0 | 0.6 | 0.5 | 2.9 | 3.6 | 5.0 | 2.4 | 8.9 | 21.1 | 8.1 | 29.4 | 13.4 |
| F2(C) | 9.9 | 0.7 | 1.5 | 0.4 | — | 1.7 | 13.7 | 1.9 | 3.6 | 38.5 | 2.6 | 24.8 | — | 0.7 |
| F3(C) | 13.4 | 3.5 | 9.3 | 3.2 | 4.4 | 24.6 | 10.5 | — | 17.5 | 10.9 | — | 2.7 | — | — |
| Cocoa butter | 1.0 | 0.2 | 2.4 | — | — | 2.2 | 18.9 | — | 2.4 | 41.3 | — | 29.7 | — | 1.9 |

[a]P, palmitate; S, stearate; O, oleate; L, linoleate.

[b]F1 fractions represent trisaturated fatty acid glycerides (PPP/PPS/SPS/SSS); F2 fractions represent disaturated-monounsaturated fatty acid glycerides (PPL/POP/POS/SOS); F3 fractions represent both monosaturated-diunsaturated fatty acid glycerides (POL/POO/SOO) and triunsaturated fatty acid glycerides (OOL/OOO).

TABLE 2

**Glyceride Distribution in Various Fractions (F1,F2,F3) of Interesterified Palm Olein and Fractional Yields Obtained by Different Solvent Fractionation Procedures (A,B,C)**

| Fraction | Yield (% wt) | Glyceride distribution (% wt, calc.) | | | | |
|---|---|---|---|---|---|---|
| | | Diglycerides | S3 | S2U | SU2 | U3 |
| F1(A) | 22.1 | 32.1 | 94.5 | 11.0 | 1.9 | 1.4 |
| F2(A) | 26.0 | 2.0 | 5.5 | 58.1 | 5.7 | 2.2 |
| F3(A) | 51.9 | 65.9 | — | 30.9 | 92.4 | 96.4 |
| F1(B) | 25.9 | 30.9 | 98.8 | 11.9 | 1.4 | — |
| F2(B) | 32.5 | 9.7 | 1.2 | 67.7 | 16.2 | 11.1 |
| F3(B) | 41.6 | 59.4 | — | 20.4 | 82.4 | 88.9 |
| F1(C) | 21.6 | 5.6 | 91.7 | 11.7 | 4.5 | 4.2 |
| F2(C) | 26.1 | 25.3 | 8.3 | 51.7 | 5.9 | 4.0 |
| F3(C) | 52.3 | 69.1 | — | 36.6 | 89.6 | 91.8 |

contained 89.0% of these butter-like triglycerides as against 89.9% in cocoa butter itself. HPLC comparison of F2(A) with natural cocoa butter showed excellent compositional matching of their corresponding glyceride components (Fig. 1). Both the F2(B) and F2(C) fractions, however, contained lower levels of the cocoa butter-like triglycerides (*ca.* 78%).

*Melting profiles.* The three fractionation procedures adopted in this study gave essentially two fat fractions (F1 and F2) that could be characterized by their distinct melting characteristics. The S3-rich F1 fractions, namely F1(A), F1(B) and F1(C), showed higher melting profiles between 45.5°C and 62.2°C, as analyzed by DSC (Fig. 2). The higher melting points of these fat fractions are largely due to their higher saturated triglyceride levels (*ca.* 66%), where every saturated triglyceride in a fat is known to contribute to increasing the melting point (10,14). A comparison of the melting characteristics of the cocoa butter-like fats, F2(A), F2(B) and F2(C), with natural cocoa butter showed these fats to have fairly comparable stable $\beta$-phase melting profiles in the physiologically important temperature range between 28.1 and 38.9°C (Fig. 3).

In conclusion, enzymatically interesterified palm olein is a good source of the unique cocoa butter-like triglycerides, which could be selectively fractionated in hexane and/or acetone to give a yield of approximately 25% of

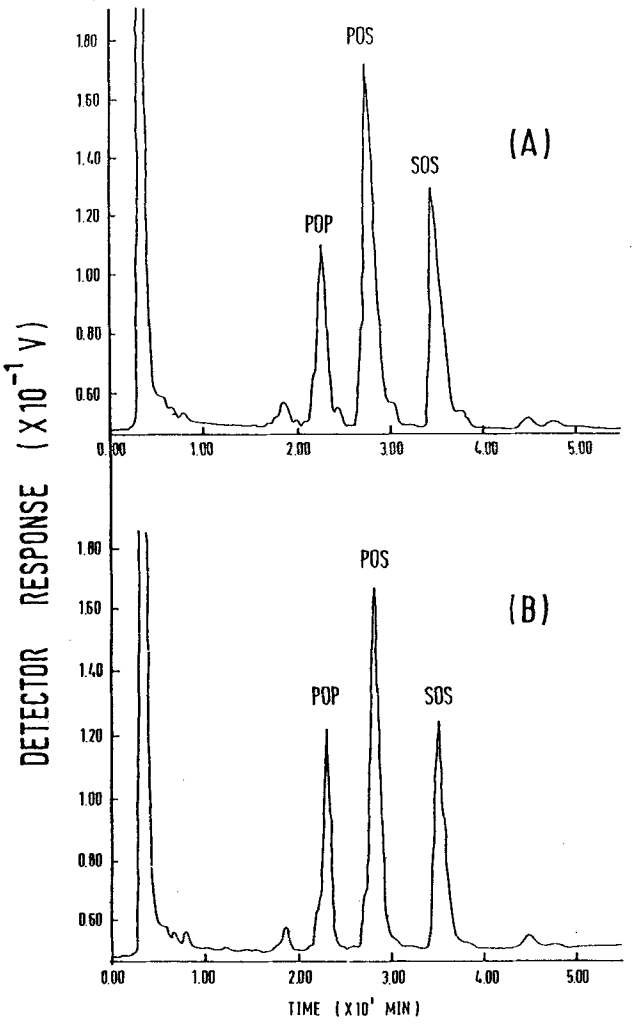


**FIG. 1. HPLC trace of cocoa butter-like fat F2(A) from palm olein (A) and cocoa butter (B); (POP) dipalmitoyl-oleoyl-glycerol, (POS) palmitoyl-oleoyl-stearoyl-glycerol, (SOS) distearoyl-oleoyl-glycerol.**

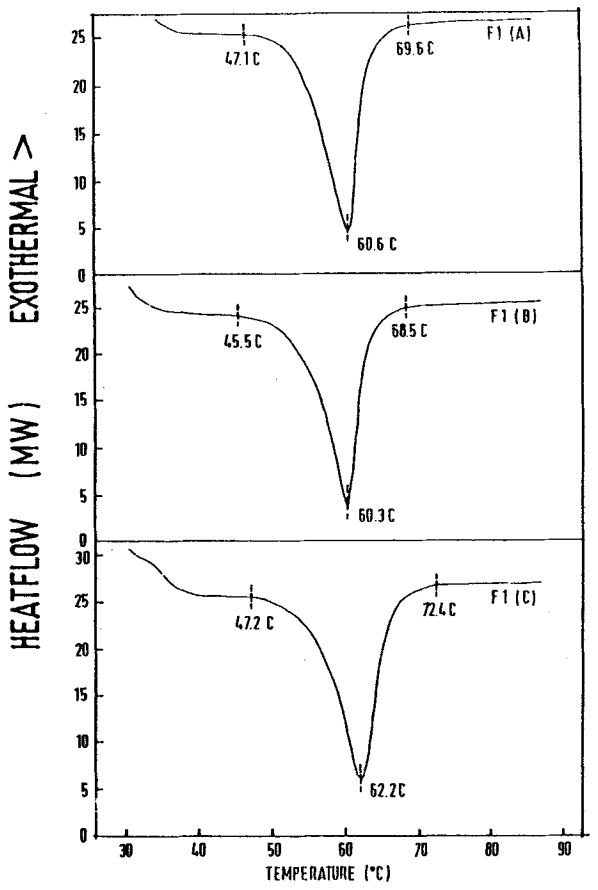


**FIG. 2. DSC analyses of high-melting fractions F1(A), F1(B) and F1(C) from interesterified palm olein.**

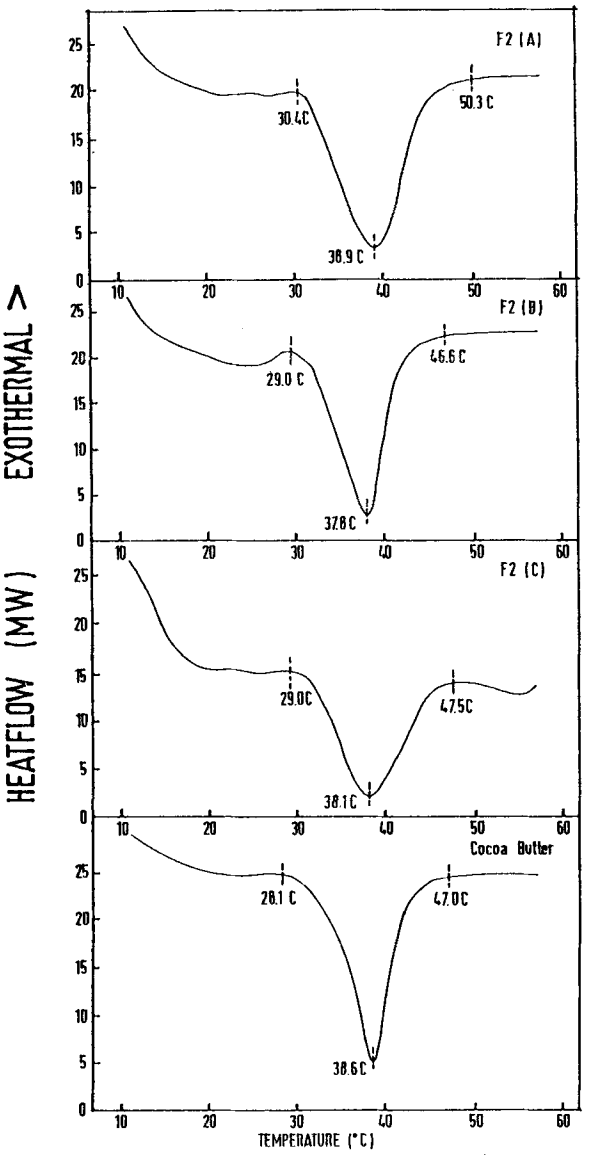


**FIG. 3. DSC analyses of cocoa butter-like fractions F2(A), F2(B) and F2(C) from interesterified palm olein and cocoa butter.**

the weight of the original palm olein. While the three solvent fractionation procedures as adopted in the present study are not able to accomplish a complete precipitation of the cocoa butter-like triglycerides, they are, nevertheless, worthy of consideration for the production of cocoa butter-like fat from palm oil.

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