PRODUCTION OF COCOA BUTTER-LIKE FATS FROM PALM OIL BY ENZYMATIC INTERESTERIFICATION

C.N. Chong and Y.M. Hoh
Standards and Industrial Research Institute of Malaysia (SIRIM)
P.O. Box 7035, 40911 Shah Alam, Selangor D. E.
C.W. Wang
Department of Biochemistry, University of Malaya
59100 Kuala Lumpur
C.K. Ooi
Palm Oil Research Institute of Malaysia (PORIM)
P.O. Box 10620, 50720 Kuala Lumpur

***RINGKASAN** : Penggunaan enzim lipase untuk memasukkan asid stearik ke dalam tiga pecahan minyak kelapa sawit - minyak sawit, stearin sawit dan olein sawit - dengan menggunakan lipase1,3-regiospecific Novo Lipase Lipozyme IM 20 menghasilkan campuran kompleks gliserida-gliserida dan asid lemak bebas. Inkorporasi stearoyl dalam olein sawit menghasilkan 40-50% trigliserida-trigliserida seperti gliserida mentega koko dalam pecahan gliserida, iaitu distearoyl-oleoyl-glycerol, palmitoyl-oleoyl-stearoyl-glycerol dan dipalmitoyl-oleoyl -glycerol. Di dalam pemecahan olein sawit yang telah diinteresterkan, asid-asid lemak bebas diasingkan daripada campuran produk melalui penyulingan stim di bawah keadaan vakum, dan kemudiannya diikuti oleh pengkristalan berperingkat gliserida-gliserida bebas dari asid lemak dalam heksana dan/atau aseton. Prosedur ini menghasilkan lemak seperti mentega koko, di mana trigliserida dan profil cairan nya adalah setanding dengan mentega koko. Keputusan ini dibuktikan melalui kromotografi cecair keupayaan tinggi fasa terbalik dan kalorimeter 'scanning differential'. Lemak seperti mentega koko yang dihasilkan adalah 26 - 32% daripada minyak yang telah diinteresterkan dan bergantung kepada prosedur penghabluran yang diamalkan.*

ABSTRACT : Lipase-catalysed incorporation of stearic acid into three RBD palm oil fractions - palm oil, palm stearin and palm olein - by the 1,3-regiospecific Novo lipase Lipozyme IM20 produced a complex mixture of fatty acid glycerides and free fatty acids. Stearoyl incorporation resulted in 40-50% of the desired cocoa butter-like triglycerides in the fatty acid glyceride portion, namely distearoyl-oleoyl-glycerol, palmitoyl-oleoyl-stearoyl-glycerol and dipalmitoyl-oleoyl-glycerol. In the fractionation of interesterified palm olein, free fatty acids were removed from the product mixture by steam distillation under vacuum followed by fractional crystallisation of the fatty acid-free glycerides in hexane and/or acetone. This procedure gave cocoa butter-like fats with triglyceride and melting profiles comparable to that of cocoa butter as adduced by reversed phase high performance liquid chromatography (HPLC) and differential scanning calorimetry (DSC), respectively. The yields of the cocoa butter-like fats ranged from 26-32% of the weight of the interesterified oil, depending on the crystallisation prŏcedures adopted.

KEYWORDS : Cocoa butter-like fats--RBD palm oil--stearic acid--interesterification--lipase--fractional crystallisation.

INTRODUCTION

There is considerable industrial potential for the application of lipases in the oils and fats industry (Macrae, 1985). A breakthrough in enzyme biotechnology allows the lipase enzyme to be bound or immobilised in a carrier system to function in non-hydrolytic processes (Posorske *et al.,* 1988). In nature the function of lipases is to hydrolyse fats by splitting the triglycerides into free fatty acids(FFA) and glycerol. The new enzyme system brings about modification of oils and fats through interesterification and ester synthesis where the oils and fats are reacted with fatty acids, alcohols or esters with the interchange of fatty acid groups to produce a new triglyceride or ester (Hansen and Eigtved, 1986). Use of immobilised lipases having regio- or stereospecificity, for interesterification of lipids such as palm oil, would produce tailor-made triglycerides which are hitherto not obtainable by normal chemical interesterification (Macrae, 1983). Products as varied as cocoa butter substitutes, speciality fats for margarine, emulsifiers, waxes, cosmetics and many others are beneficiaries of the new immobilised lipase system.

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 crystallisation and melting characteristics so essential in providing in chocolate confectionery, a sharp melting in the region of body temperature (Coleman *et al.*, 1980; Macrae, 1985). However, there are a number of fats suitable for partial or total 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 (Bloomer *et al.*, 1990; Coleman *et al.*, 1980; Matsuo *et al.*, 1981; Okawachi and Sagi, 1985; Pease, 1985).

Interesterification of palm oil with stearic acid will result 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 FFA from the glycerides and the latter into the desired cocoa butter components. Steam fractionation under vacuum is a distillation process which could lead to the complete physical separation of lower boiling fractions, such as FFA, from higher boiling components such as oils and fats. The FFA-free glyceride fraction can then be subjected to conventional fat fractionation techniques such as countercurrent liquid-liquid extraction and crystallisation from solvents to produce fats from an oil with distinct melting profiles (Chang *et al.*, 1990; Deffense, 1985; Macrae, 1983). Pszczola (1991), 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 filtered out of the oils. The process is repeated at lower temperatures so that different crystals of the solid fat particles are further solidified and filtered out. In general, the process involves

refrigeration, solvent fractionation, evaporation and solvent recovery.

This paper presents the findings of the batch enzymatic interesterification (acidolysis) of three refined, bleached and deodorised (RBD) palm oil fractions, namely palm oil, palm stearin and palm olein with stearic acid by the 1,3-specific Novo immobilised lipase Lipozyme IM20 with the view of producing a cocoa butter equivalent. Specifically, it will describe the various fractionation steps based on the above fractionation concepts for obtaining the desired cocoa butter-like fats from interesterified palm olein and the characterisation of the various fat/oil fractions in terms of their diglyceride, triglyceride and melting profiles.

MATERIALS AND METHODS

Immobilised Lipase

The 1,3-specific Novo lipase Lipozyme IM 20 (Activity 28.1 BIU/g) was a preparation of *Mucor miehei* lipase (E.C. 3.1.1.3) immobilised on a macroporous anion exchange resin (donated by Novo Nordisk A/S Malaysia). It is optimally used at temperatures between 60°C and 70°C.

Palm Oil Fractions

RBD palm oil, palm stearin and palm olein were obtained from a local palm oil refinery. 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. (Deffense, 1985)

Cocoa Butter

Prime processed cocoa butter from selected Malaysian cocoa beans was obtained from a local cocoa butter producer.

Chemicals

Reagent-grade stearic acid (97.3%) was supplied by Riedel-Dehaen A.G. Seelze-Hannover. An industrial-grade stearic acid (98.3%) sample derived from palm oil was donated by a local oleochemical manufacturer. All chemicals and solvents used were obtained commercially and were of the highest purity available. The lipid standards were obtained from Sigma Chemical Company (USA) or by synthesis.

Enzymatic Interesterification

Batch enzymatic interesterification of the various palm oil fractions with stearic acid was carried out in closed flasks under the following experimental conditions: (A). at 30°C for 3 days in a solvent mixture of hexane/diethylether 4:1 (v/v) (500% v/w oil) in the presence of Lipozyme (1% w/w oil) and water (1% v/w oil) with orbital shaking at 160 rpm, and (B). stirred at 60°C for 20 h in the presence of Lipozyme (10% w/w oil) and water (1% v/w oil) without the use of solvents.

Condition A was adopted for the acidolysis of all the palm oil fractions using an oil-stearic acid weight ratio of 1:1. Both conditions A and B were adopted for a specific acidolysis study of palm olein with stearic acid using weight ratios of 2:1 and 10:3 respectively.

A batch solvent-free enzymatic interesterification of palm olein (1 kg) with industrial-grade stearic acid (0.5 kg) was also carried out in a closed 5-L glass reactor equipped with a mechanical stirrer (200 rpm) and heater (60°C) for 20 h 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 immobilised 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 h to give a steam-distilled fatty acid distillate (40.3%; 91.9% FFA) and the residual solid glyceride fraction (59.7%; 0.04% FFA). Solvent fractionation of the steam-fractionated glyceride fraction was carried out using 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 h. The precipitated fat F1(A) was filtered off and the filtrate evaporated to dryness. The mother liquor was then dissolved in acetone (1:10 w/v) and cooled at 4°C/24 h. A second precipitated fat fraction F2(A) was obtained. The solvent was evaporated off from the resultant mother liquor to yield an oil fraction F3(A).

Procedure B: The above solvent fractionation procedure was repeated but using instead 1:5 (w/v) glyceride-hexane and 1:5 (w/v) mother liquor-acetone crystallisation systems 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 h to give a fat fraction F1(C) which was filtered off and the filtrate was then cooled at 4°C/24 h to give another fat fraction F2(C). The final filtrate was evaporated to dryness to give an oil fraction F3(C).

Analysis

Glyceride Composition

The interesterified triglycerides derived from the various palm oil fractions were separated from the reaction mixture by column chromatography using alumina as the adsorbent and petroleum ether (40-60°C) as the eluting solvent. The fatty acyl groups in the various palm oil fractions and their resultant interesterified triglycerides were determined as their methyl esters by GLC after base-catalysed transesterification with 0.5 M sodium methylate. GLC analysis was carried out using a Perkin Elmer Sigma 2000 Chromatograph equipped with a FID and integrator. The methyl esters were separated isothermally at 180°C in a glass column of 5% DEGS on 100-120 mesh diatomite C/AW or at 195°C in a glass column of 10% SP-2300 on 100-120 mesh chromosorb WAW. The flow rate of the carrier gas (N_2) was 20 $ml min^{-1}$.

The total acyl carbon number of the palm oil fractions and their resultant interesterified triglycerides were determined by GLC using a packed glass column (2' x 1/4") of 3% OV - 1 on 100-120 mesh Supelcoport (Supelco). Column temperature was programmed from 325-345°C at 2°C per min with a N_2 flow rate of 40 $ml min^{-1}$.

The diglyceride and triglyceride components were identified by HPLC using a Waters HPLC System equipped with a RI 410 refractometer detector. A combination of Supelco LC 18 (25 cm x 4 mm i.d.) and Nova-Pak C 18 (10 cm x 8 mm) columns were connected in series using a mobile phase of 63.6 : 36.4 (v/v) acetone and acetonitrile at a flow rate of 1 $mlmin^{-1}$. Each sample was dissolved in tetrahydrofuran to make a 5% solution. The injection was 20 µl per injection using an auto-injector. Both detector and columns were maintained at 35°C.

Residual Free Fatty Acids

The residual free fatty acids after the interesterification reaction were determined by titration with 0.1M KOH using phenolphthalein as the indicator. The fatty acid profile of the acids was determined by GLC after their separation from the reaction mixture by silica gel TLC using a hexane-diethylether-acetic acid (85:25:1, v/v/v) developing system, followed by methylation with methanol-sulphuric acid to their corresponding methyl esters.

Melting Profiles

The melting characteristics of the various fat fractions were analysed by a Mettler TA 4000 Differential Scanning Calorimeter.

RESULTS AND DISCUSSION

Incorporation of Stearic Acid into Palm Oil Fractions

The fatty acyl groups in the various RBD palm oil fractions, namely palm oil, palm stearin and palm olein, the interesterified fats (S) and cocoa butter were compared (Table 1). The most notable difference between the palm oil fractions and cocoa butter was found in their stearoyl (C18:0) content where about 5% was present in the former and 36% in the latter. The average palmitoyl (C16:0), oleoyl (C18:1) and linoleoyl (C18:2) contents of the palm oil fractions were higher at 46%, 40% and 8% respectively as against 27%, 34% and 3% for the corresponding groups in cocoa butter. The higher degree of saturation in cocoa butter (63%) as compared to that in palm stearin (59%) and olein (46%) generally confers the solid characteristics in the former. Therefore, to develop a suitable cocoa butter equivalent, the stearoyl content and degree of saturation in the RBD palm oil fractions must be increased with a proportionate decrease in the palmitoyl, oleoyl and linoleoyl contents.

There was substantial incorporation of stearic acid into the various RBD palm oil fractions, with a concomitant decrease in the palmitoyl and oleoyl contents after batch enzymatic interesterification of the oils (1 part) with stearic acid (1 part) in diethylether-hexane. However, no significant change in the overall linoleoyl content was observed. The stearoyl contents of the interesterified triglycerides which ranged from 29.6-33.4% were in close proximity to that of cocoa butter (36.3%). The average fatty acyl profile of the interesterified triglycerides from the three RBD palm oil fractions was generally

TABLE 1. FATTY ACYL PROFILES OF RBD PALM OIL FRACTIONS, THEIR INTERESTERIFIED PRODUCTS (FROM 1:1, OIL-STEARIC ACID MIX IN SOLVENTS, S-TYPE) AND COCOA BUTTER

	Fatty Acid (%)						
	Lauric (12:0)	Myristic (14:0)	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Others
Palm Oil	0.2	1.0	43.9	4.5	41.4	8.9	0.1
Palm Stearin	0.2	1.0	53.4	4.5	33.3	7.1	0.5
Palm Olein	0.2	0.8	40.3	5.0	44.6	8.8	0.3
Modified P. Oil (S)	0.1	0.7	26.9	32.8	32.1	6.9	0.5
Modified P. Stearin (S)	0.2	1.2	35.3	29.6	28.0	5.4	0.3
Modified P.Olein (S)	0.2	0.8	22.9	33.4	34.4	7.4	0.9
Cocoa Butter	-	0.1	26.6	36.3	34.1	2.6	0.3

comparable to that of cocoa butter with C16:0 (28.4% vs 26.6%), C18:0 (31.9% vs 36.3%) and C18:1 (31.5% vs 34.1%). The interesterified products had approximately 61% saturated fatty acyl content in the mix.

Table 2 shows the results of the interesterification of RBD palm olein with stearic acid using varying oil-stearic acid weight ratios under two prescribed experimental conditions A using a solvent mix and B without use of solvents.

The study showed that both conditions gave comparable results as judged by the fatty acyl profiles of the resultant interesterified triglycerides for a particular oil-stearic acid mix. The solvent-free B, 1:1 oil-stearic acid mix study was abandoned because of poor solubility of the stearic acid in palm olein and its high viscosity. Both the 1:1 and 2:1 oil-stearic acid mixes in solvents gave comparable fatty acyl profiles in the interesterified triglycerides. Concomitant with the solvent-free 2:1 oil-stearic acid mix, the average fatty acyl profile of their resultant triglycerides was comparable to that of cocoa butter with C16:0 (22.7% vs 26.6%), C18:0 (36.4% vs 36.3%) and C18:1 (33.4% vs 34.1%). The fatty acyl profile of the interesterified triglycerides obtained from the 10:3 oil-stearic acid mix under both experimental conditions showed a lower stearic acid content (25.8%) in the fat. The compositional changes of the fatty acyl groups during batch enzymatic interesterification of the solvent-free 2:1 and 10:3 palm olein-stearic acid mixes are as shown in Figures 1 and 2 respectively. Essentially, complete interesterification as evidenced by stearate incorporation was obtained after about 8 h. No additional incorporation was observed even at 20h.

TABLE 2. COMPOSITION OF FATTY ACYL GROUPS IN TRIGLYCERIDES OBTAINED AFTER BATCH ENZYMATIC INTERESTERIFICATION OF PALM OLEIN WITH STEARIC ACID UNDER EXPERIMENTAL CONDITIONS A AND B

RBD Palm Olein : Stearic Acid	Experimental Conditions A or B	Fatty Acid (%) in Interesterified Triglycerides						
		12:0	14:0	16:0	18:0	18:1	18:2	Others
1 : 1	A	0.2	0.8	22.9	33.4	34.4	7.4	0.9
2 : 1	A	0.1	1.0	22.0	39.5	32.8	2.9	1.7
2 : 1	B	0.1	0.9	23.1	36.3	32.9	5.8	0.9
10 : 3	A	0.1	0.8	26.8	24.4	39.7	7.8	0.4
10 : 3	B	0.2	0.8	26.4	27.2	38.8	6.1	0.5

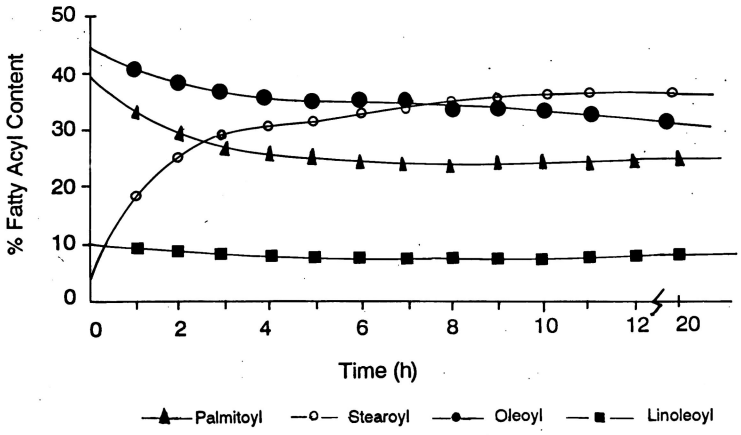

Figure 1. *Fatty acyl profile of products during enzymatic interesterification of palm olein (2 parts) with stearic acid (1 part)*

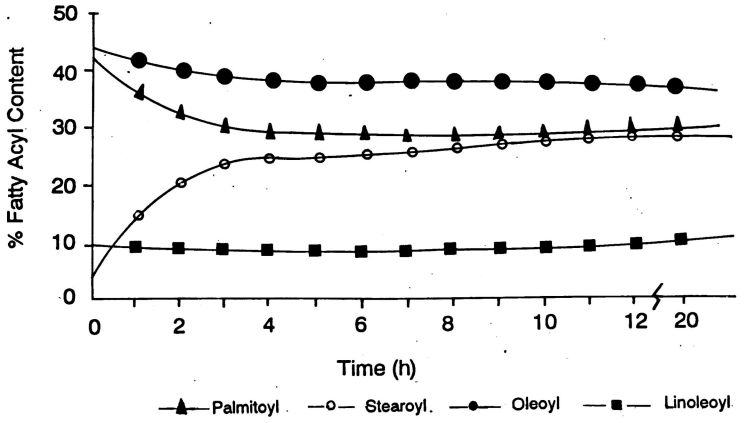

Figure 2. *Fatty acyl profile of products during enzymatic interesterification of palm olein (10 parts) with stearic acid (3 parts)*

Table 3 shows the compositions of the various fatty acyl groups in the reactants and products of the solvent-free palm olein-stearic acid (2:1) reaction mix. The molar ratio of residual free fatty acids after interesterification to the free fatty acids in the reactants was found to be 1.2. A mass balance analysis showed that the total acylglyceride yield was 90% with the 10% loss probably attributed to hydrolysis of the original oil. This was confirmed by fractionation of the total products by steam distillation under vacuum into the respective glyceride (59.7%) and the free fatty acid distillate (40.3%) fractions. The fatty acyl profiles of the total acylglyceride and triglyceride were fairly identical reflecting minimal formation of mono- and diglycerides during interesterification.

Triglyceride Composition

Total acyl carbon number profile

Table 4 shows the distribution of triglycerides in terms of their total acyl carbon number in the various palm oil fractions, interesterified products obtained in solvent free 2:1 oil-stearic acid reaction mixes and cocoa butter. All the palm oil fractions showed high levels of C50 (42.5-44.0%) and C52 (29.9-43.4%) and relatively low C48 (3.4-17.6%) and C54 (5.6-6.7%). Their interesterified triglycerides showed reduced levels of C50 (17.9-27.1%) and a concomitant increase in C54 (21.9%). The levels of the C52 triglycerides in the modified palm oil fractions remained high (41.3-44.8%). The mean acyl carbon number profile of the interesterified triglycerides from the three palm oil fractions, however, was fairly comparable to that of cocoa butter with a fairly good fit at C50 (22% vs 19%), C52 (43% vs 48%) and C54 (28% vs 30%).

Triglyceride types

Cocoa butter is composed of three predominant fatty acids, namely palmitic, stearic and oleic acids, which together account for over 95% of the fatty acids present. A unique positioning of these fatty acids with saturated fatty acids in the 1,3-positions and the unsaturated

TABLE 3. FATTY ACYL GROUPS IN REACTANTS AND PRODUCTS OBTAINED AFTER BATCH ENZYMATIC INTERESTERIFICATION OF PALM OLEIN (10 GM) WITH STEARIC ACID (5 GM) UNDER SOLVENT-FREE EXPERIMENTAL CONDITIONS

Reactants/Products	Fatty Acid (%)						
	12:0	14:0	16:0	18:0	18:1	18:2	Others
RBD Palm Olein	0.2	0.8	40.3	5.0	44.6	8.8	0.3
Stearic Acid	-	0.1	1.4	97.3	-	-	1.2
Total Acylglycerides	0.3	1.4	23.6	34.2	33.3	7.0	0.2
Total Triglycerides	0.1	0.9	23.1	36.3	32.9	5.8	0.9
Residual Free Fatty Acids	0.4	0.6	28.5	44.6	21.0	4.5	0.4

TABLE 4. TRIGLYCERIDE CARBON NUMBER PROFILES OF RBD PALM OIL FRACTIONS, THEIR MODIFIED PRODUCTS AND COCOA BUTTER

	Total Carbon Number %					
	C46	C48	C50	C52	C54	Others
Palm Oil	0.5	8.8	42.5	38.3	6.3	3.6
Palm Stearin	0.9	17.6	44.0	29.9	5.6	2.0
Palm Olein	0.1	3.4	43.1	43.4	6.7	3.3
Modified P. Oil	0.3	5.1	21.1	41.3	28.7	3.5
Modified P. Stearin	0.3	7.1	27.1	43.5	21.9	0.1
Modified P. Olein	0.1	3.5	17.9	44.8	33.6	0.1
Cocoa Butter	-	0.1	18.6	48.1	30.5	2.7

fatty acid in the 2-position gives rise to the desirable characteristics inherent in cocoa butter. These mono-oleo disaturates are predominantly 1,3-distearoyl-2-oleoyl glycerol (SOS), 1,3-dipalmitoyl-2-oleoyl-glycerol (POP) and 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS) which together account for about 80% of the triglyceride composition of cocoa butter (Shukla, 1988).

A comparison of the triglyceride types present in the palm oil fractions and their interesterified products with cocoa butter is shown in Table 5. The palm oil fractions were particularly rich in POP (33.9-41.4%)

TABLE 5. COMPARISON OF TRIGLYCERIDE COMPOSITON OF RBD PALM OIL FRACTIONS, THEIR MODIFIED FATS AND COCOA BUTTER

	OOL	POL	PPL	OOO	POO	POP	PPP	SOO	POS	PPS	SOS	SPS	SSS
Palm Oil	1.3	10.0	11.4	2.9	24.7	33.9	6.5	2.6	5.6	1.1	-	-	-
Palm Stearin	0.5	8.8	9.8	2.6	19.4	36.9	13.6	1.2	5.2	2.0	-	-	-
Palm Olein	0.9	6.4	7.4	4.9	28.5	41.4	-	4.3	6.2	-	-	-	-
Modified Palm Oil	1.4	3.3	0.4	5.8	13.2	11.5	4.1	9.5	19.0	11.3	9.6	9.5	1.4
Modified Palm Stearin	2.5	4.6	2.6	3.8	11.9	12.7	5.3	7.1	18.5	13.7	8.1	8.2	1.0
Modified Palm Olein	1.7	4.6	2.7	2.4	14.5	12.3	1.0	11.4	22.8	7.2	12.1	5.3	2.0
Cocoa Butter	-	1.2	0.9	-	2.5	18.4	-	-	44.4	-	30.8	-	1.8

(P Palmitate; S Stearate; O Oleate; L Linoleate)

but low in POS (2.0-6.2%) with a negligible amount of SOS. Furthermore, they contained high levels of POL (6.4-10.0%), PPL (7.4-11.4%), POO (19.4-28.5%) and, except for palm olein, PPP (6.5-13.6%). The modified fats showed reduced levels of POP (11.5-12.7%) and increased levels of POS (18.5-22.8%) and SOS (8.1-12.1%).

Table 6 shows a comparison of the interesterified palm oil fractions obtained in the present study with those obtained by Macrae (1985) and Matsuo *et al.* (1981) from palm oil midfraction and fractionated palm oil, respectively. While the latter investigators obtained fractionated interesterified products closely resembling cocoa butter, our study showed that the interesterified palm oil fractions, without prior fractionation, contained a very complex mixture of triglycerides which was high in trisaturated (PPP,PPS,SPS,SSS) and monosaturated-diunsaturated (POL,POO,SOO) fatty acid glycerides. Overall, the stearoyl incorporation in the various palm oil fractions resulted in the formation of some 40-50% of the desired disaturated-monounsaturated fatty acid glycerides (SOS, POS, POP) whose relative compositions were comparable to that of cocoa butter.

The remainder of the modified fats were made up of approximately equal proportions of the trisaturated and monosaturated-diunsaturated glycerides. These triglycerides are considered as undesirable components in cocoa butter

TABLE 6. A COMPARISON OF THE DISTRIBUTION OF TYPES OF TRIGYCERIDES IN INTERESTERIFIED PALM OIL FRACTIONS AND COCOA BUTTER

	Types of Triglycerides (%)					S2U (%)			
	S3	S2U	SU2	U3	Others	SOS	POS	POP	Others
Modified P. Oil[a]	26.3	40.5	26.0	7.2	-	23.7	46.9	28.4	1.0
Modified P. Stearin[a]	28.2	41.9	23.6	6.3	-	19.3	44.1	30.3	6.3
Modified P. Olein[a]	15.5	49.9	30.5	4.1	-	24.2	45.7	24.6	5.5
Interesterified P. Oil Fraction[b]	3.0	91.5	4.0	-	1.5	31.1	42.4	17.7	8.8
Interesterified P. Oil Fraction[c]	1.5	83.2	13.0	2.3	-	30.1	49.3	19.8	0.8
Cocoa Butter	1.8	94.5	3.7	-	-	32.6	47.0	19.5	0.9

P Palmitate; S Stearate; O Oleate; L Linoleate
S3 : Trisaturated Fatty Acid Glycerides (PPP/PPS/SPS/SSS);
S2U: Disaturated - Monounsaturated Fatty Acid Glycerides (PPL/POP/POS/SOS);
SU2: Monosaturated - Diunsaturated Fatty Acid Glycerides (POL/POO/SOO);
U3 : Triunsaturated Fatty Acid Glycerides (OOL/OOO)

[a] Present study
[b] Macrae, A.R. (1985): Interesterification of a mixture of palm oil midfraction (1 part) and stearic acid (0.4 part) in petroleum ether using *Mucor miehei* lipase catalyst in a packed bed reactor at 40°C/10 min RT.
[c] Matsuo *et al.* (1981): Interesterification of a mixture of fractioned palm oil (1 part) with methyl stearate (1 part) using an immobilized lipase of *Rhizopus niveus* at 40°C/72 h.

equivalents as they adversely affect the melting point and solid fat content, and hence their quality (Moore *et al.*, 1986; Pease, 1985). The formation of trisaturated glycerides is believed to be due to acylation with saturated fatty acids of the palm oil fractions with saturated fatty acids in the 2-position and acyl migration in diglycerides formed from the triglycerides during the interesterification reaction (Bloomer *et al.* 1990; Macrae, 1983). Thus, the direct attainment of the desirable levels of mono-oleo disaturates as required for cocoa butter production would be complicated by the presence of the complex mix of triglycerides in the original palm oil fractions which would be subjected to the same competing 1,3-regiospecific interesterification reactions operative in the reaction system. The formation of high levels of the trisaturated and monosaturated-diunsaturated fatty acid glycerides is a consequence of these competing reactions. Thus, appropriate fractionation procedures will have to be devised to separate the desired cocoa butter equivalent components from the resultant complex interesterified palm oil fractions. Conventional fat fractional techniques such as countercurrent liquid-liquid extraction and crystallisation from solvents had been proposed (Chang *et al.*, 1990; Deffense, 1985; Macrae, 1983).

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 (40.3%; 91.90% FFA) and the residual solid glyceride fraction (59.7%; 0.04% FFA).

Table 7 shows a comparison of the diglyceride and triglyceride compositions by HPLC of the interesterified palm olein fraction and the various fractions obtained therefrom by hexane and/or acetone fractionation. The distribution of the diglycerides and types of triglycerides (S3, S2U, SU2 and U3) in the various fractions is as indicated in Table 8. The interesterified palm olein (IPO) contained 10.2 % of diglycerides with the rest made up of thirteen chromatographically detectable triglycerides. The stearoyl incorporation had resulted in the formation of about 40% of the cocoa butter-like fat, the disaturated-monounsaturated fatty acid glycerides (S2U), namely palmitoyl-oleoyl-stearoyl (POS), dipalmitoyl-oleoyl-glycerol (POP) and distearoyl-oleoyl-glycerol (SOS).

The S3 (PPP/PPS/SPS/SSS)-rich fat fractions, F1(A), F1(B) and F1(C), contained the bulk of the S3-glycerides (91.7-98.8%) of the starting IPO. Some 11.0-11.9% of the cocoa butter-like fat components (S2U) were, however, co-crystallised along with these fractions. Their overall fractional yields ranged from 21.6-25.9%.

The S2U (PPL/POP/POS/SOS)-rich fractions, F2(A), F2(B) and F2(C), contained 51.7-67.7% of the total cocoa butter-like glycerides present in the IPO. Of particular interest is the F2(A) fraction which contained 89.0% of POP/POS/SOS as against 89.9% in cocoa butter. HPLC comparison of F2(A) with authentic cocoa butter showed excellent compositional matching of their corresponding glyceride components

TABLE 7. COMPARISON OF DIGLYCERIDE AND TRIGLYCERIDE COMPOSITION OF VARIOUS FRACTIONS (F1, F2, F3) OF INTERESTERIFIED PALM OLEIN OBTAINED BY DIFFERENT FRACTIONATION PROCEDURES (A, B, C)

Fraction	Diglycerides (%)	Triglycerides (%)												
		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
S3 - Rich:														
F1(A)	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
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
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
S2U - Rich:														
F2(A)	0.9	0.4	1.4	0.1		2.3	15.3	1.0	2.9	44.1	-	29.6	0.5	1.5
F2(B)	3.3	0.7	2.1	2.5	0.8	6.0	16.0	-	6.1	38.7	-	23.1	-	0.7
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
	(1.0)[a]	(0.2)	(2.4)	(-)	(-)	(2.2)	(18.9)	(-)	(2.4)	(41.3)	(-)	(29.7)	(-)	(1.9)
SU2-U3-Rich:														
F3(A)	14.3	3.8	9.8	3.4	4.8	25.9	9.7	-	17.7	8.7	-	1.9	-	-
F3(B)	15.6	4.1	10.4	3.4	5.2	26.8	7.8	-	19.0	6.2	-	1.5	-	-
F3(C)	13.4	3.5	9.3	3.2	4.4	24.6	10.5	-	17.5	10.9	-	2.7	-	-

P Palmitate; S Stearate; O Oleate; L Linoleate
S3 : Trisaturated Fatty Acid Glycerides (PPP/PPS/SPS/SSS);
S2U : Disaturated - Monounsaturated Fatty Acid Glycerides (PPL/POP/POS/SOS);
SU2 : Monosaturated - Diunsaturated Fatty Acid Glycerides (POL/POO/SOO);
U3 : Triunsaturated Fatty Acid Glycerides (OOL/OOO);
a : Values for Cocoa Butter in parenthesis.

TABLE 8. DISTRIBUTION OF DIGLYCERIDES AND TYPES OF TRIGLYCERIDES IN VARIOUS FRACTIONS (F1, F2, F3) OF INTERESTERIFIED PALM OLEIN OBTAINED BY DIFFERENT FRACTIONATION PROCEDURES (A, B, C)

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

(Figure 3). Both the F2(B) and F2(C) fractions, however, contained lower levels of the cocoa butter-like fat triglycerides (78%/77%). The overall yields of these cocoa butter-like fats ranged from 26.0-32.5% of the weight of the IPO.

The SU2 (POL/POO/SOO)/U3 (OOL/OOO)-rich fractions, F3(A), F3(B) and F3(C), representing 41.6-52.3% of the IPO, contained the bulk of the SU2-(82.4-92.4%), U3-(88.9-96.4%) and di-(59.4-69.1%) glycerides. Some 20.4 to 36.6% of the total cocoa butter-like fat components (S2U) were still retained in these oil fractions. The S3-glycerides were, however, absent in these fractions.

Melting Profiles

The three fractionation procedures adopted in this study gave essentially two fat fractions, F1 and F2, each having distinct melting characteristics. The S3-rich F1 fractions, namely F1(A), F1(B) and F1(C), showed higher melting files ranging between 45.5-62.2°C with each exhibiting a sharp endothermic peak centered at 60.6, 60.3 and 62.2°C, respectively, as analysed by DSC (Figure 4). F1(C), containing the highest level of trisaturated glycerides (68.9%), exhibited a slightly higher melting profile than that of F1(A) or F1(B). The higher melting points of these fat fractions are due largely to their higher saturated triglyceride levels, where every saturated triglyceride in a fat is known to contribute to making the melting point higher (Chang *et al.*, 1990).

Figure 5 shows a comparison of the melting profiles of the cocoa butter-like fats, F2(A), F2(B) and F2(C), with cocoa er. The cocoa butter-like fats showed fairly comparable melting profiles between 28 and 50°C with the former three fats showing endothermic peaks centered at 38.9, 37.8 and 38.1°C, respectively, and the cocoa butter at 38.6°C.

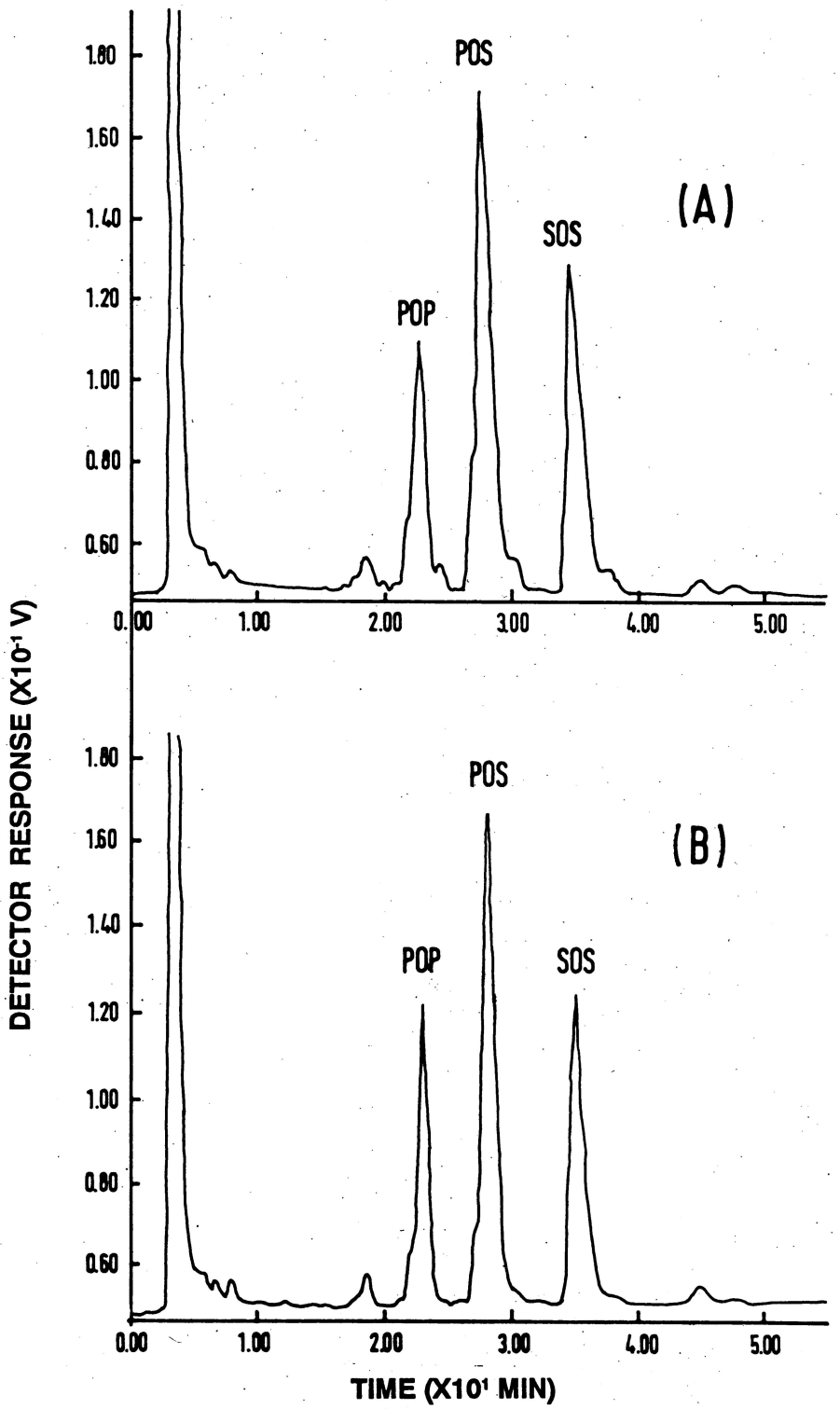

Figure 3. *HPLC chromatograms of cocoa butter-like fat F2(A) from palm olein (A) and cocoa butter (B)*

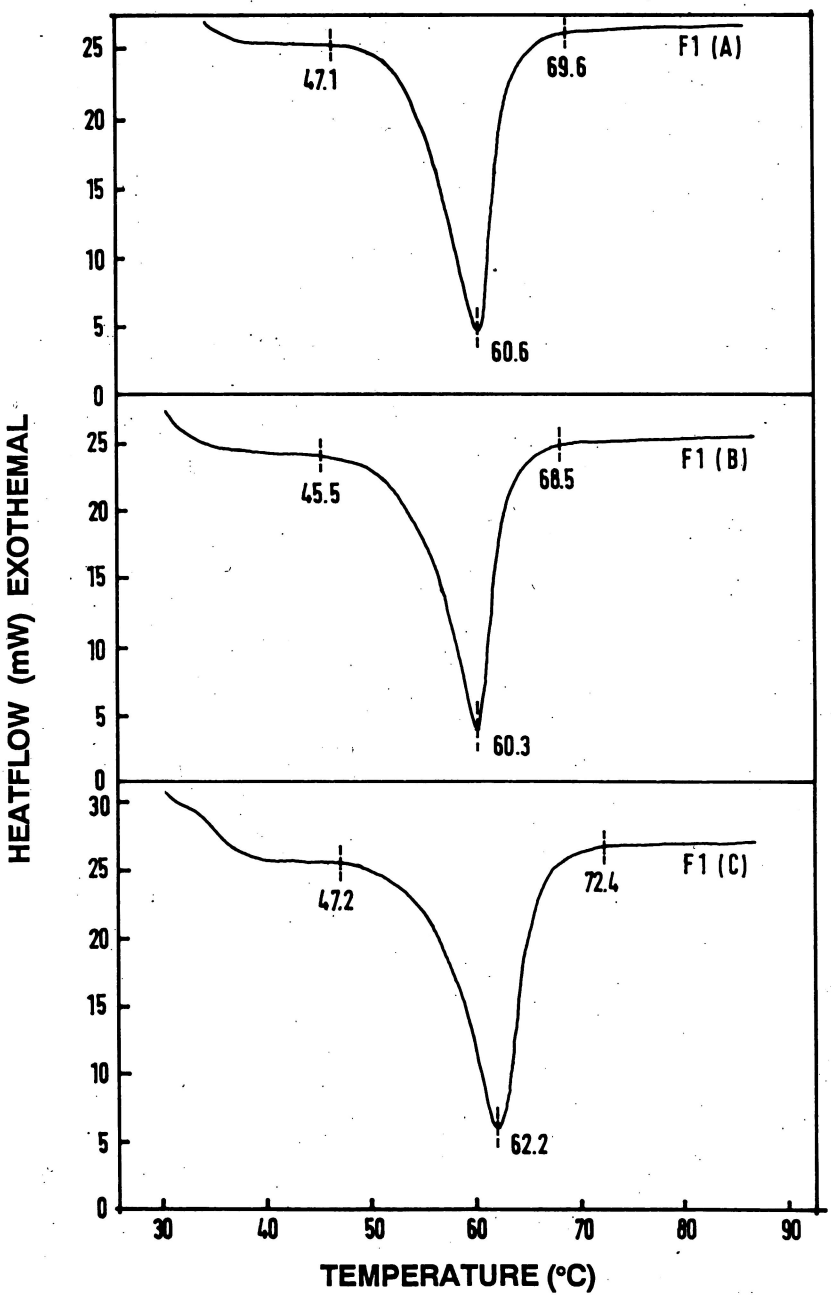

Figure 4. DSC analyses of high melting fractions F1 (A), F1 (B), and F1 (C) from interesterified palm oil

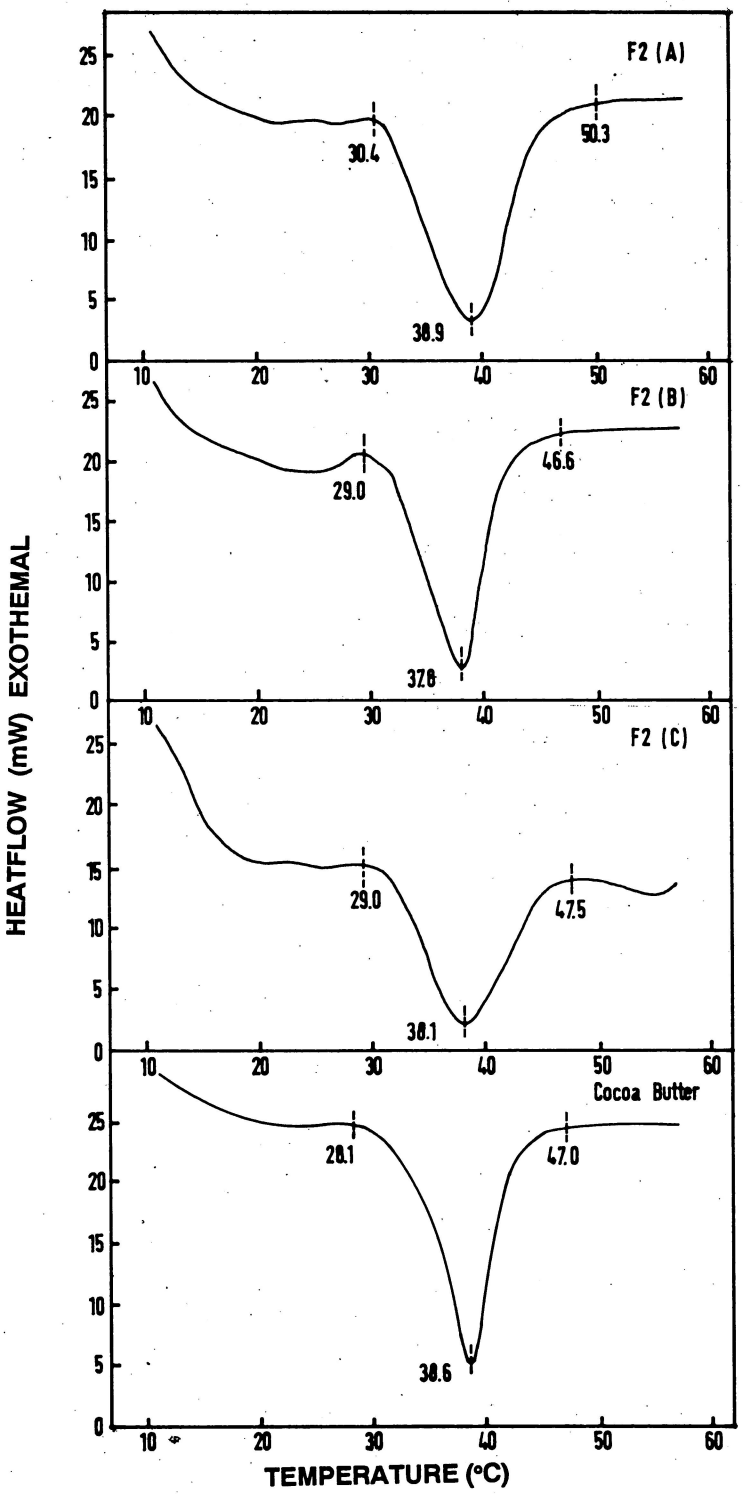

Figure 5. DSC analyses of cocoa butter

CONCLUSIONS

This investigation showed that while the various biomodified palm oil fractions had triglyceride fatty acyl and total carbon number profiles comparable to that of cocoa butter, a detailed analysis of their triglyceride compositions by high performance liquid chromatography (HPLC) indicated that overall their palmitic-oleic-palmitic (POP), palmitic-oleic-stearic (POS) and stearic-oleic-stearic (SOS) components were low at 12.2%, 20.1% and 9.9% as against 18.4%, 44.4% and 30.8% respectively for cocoa butter. While the levels of these mono-oleo disaturates were not directly attained in the interesterification reaction, nevertheless a very substantial incorporation of stearic acid had been effected by the 1,3-specific lipase-catalysed interesterification reaction resulting in the formation of 40-50% of these desirable triglycerides, whose relative compositions were comparable to that of cocoa butter. These biomodified palm oil fractions could serve as sources of the unique cocoa butter glycerides as evidenced by their selective precipitation in solvents, resulting in yields of 26-32% of the weight of the interesterified palm oil.

Thus, the three solvent fractionation procedures as adopted in the present study are worthy of consideration for the production of cocoa butter-like fats from palm oil.

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