As. J. Food Ag-Ind. **2009**, 2(04), 807-816

OPEN ACCESS

Asian Journal of Food and Agro-Industry
ISSN 1906-3040
Available online at www.ajofai.info

Research Article

Enzymatic interesterification of palm oil midfraction blends for the production of cocoa butter equivalents

S. Soekopitojo*, P. Hariyadi, T. R. Muchtadi and N. Andarwulan

Department of Food Science and Technology, Faculty of Agricultural Technology and Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Centre, Bogor Agricultural University, Bogor, Indonesia.

*Author to whom correspondence should be addressed, email: soenar_24@yahoo.com

This paper was originally presented at Food Innovation Asia, Bangkok, Thailand, August 2009.
Recieved 23 June 2009, Accepted 21 January 2010

Abstract

Interesterification of fat blends containing palm oil midfraction (PMF) and fully hydrogenated soybean oil (FHSO) at various weight ratios, catalyzed by an immobilized Thermomyces lanuginosa lipase (Lipozyme TL IM), was studied for production of cocoa butter equivalents (CBEs). CBEs have chemical and physical properties compatible with those of cocoa butter (CB) and can be used to replace CB in chocolate confectionery product. The CBEs were isolated from the crude interesterification mixture by fractional crystallization in organic solvent. Triacylglycerol (TAG) composition of the starting blends, the interesterified blends and the fat products were analyzed by reversed-phase high performance liquid chromatography (RP-HPLC) in combination with refractive index (RI) detector. Enzymatic interesterification of the substrates resulted in the formation of a complex mixture of acylglycerols and free fatty acids. Concentration of several TAGs were increased, some were decreased, and several new TAGs were formed. The main TAGs of PMF (POP, POO) and TAGs of FHSO (PSS, SSS) were decreased, whereas the desired CB TAGs (POS, SOS) were increased. Our research indicated that acyl exchange occured mainly between the palmitoyl group from the PMF and the stearoyl group from the FHSO. Fractional crystallization of the fatty acid-free acylglycerols in hexane and acetone gave the fat products whose their TAG distributions were comparable to that of CB but that they also contain diacylglycerol (DAG). The substrate weight ratio of PMF to FHSO to produce a fat product containing the highest major TAGs component of CB (POS and SOS) was 1:2.

Keywords: Enzymatic interesterification, palm oil midfraction, triacylglycerol, cocoa butter equivalents, Indonesia

Introduction

Cocoa butter (CB) is an ideal fat that contributes to the desirable textural and sensory properties of chocolate and confectionery products. CB has unique triacylglycerol (TAG) composition which provide its desirable physical properties. At below room temperature (26°C), CB is hard and brittle, but when eaten it melts completely in the mouth with a smooth, cool sensation [1]. CB is composed of predominantly (>75%) symmetrical TAG with oleic acid in the-2 position, namely palmito-oleo-stearin (POS, 36-42%), stearo-oleo-stearin (SOS, 23-29%), and palmito-oleo-palmitin (POP, 13-19%) [2].

Considering the fact that CB is a relatively costly raw material, that its quality varies, that the products containing CB has limited gloss retention and may melt in hot climates, encouraged the confectioners to seek alternatives or substitutes for CB, such as cocoa butter equivalents (CBEs) [3]. CBEs have chemical and physical properties compatible with those of CB and can be used to replace CB in chocolate confectionery products. Much attention has been given to the production of cocoa butter-like fats from fats and oils of lower value *via* interesterification with lipase [4-9], but enzymatic synthesis of cocoa butter equivalents is still of great interest in the oil and fats industry. Interesterification is an acyl-arrangement reaction. It is one of the four modification processes to alter the physico-chemical characterisitics of oils and fats, the others being blending, fractionation and hydrogenation [10].

There are a number of fats suitable for total or partial replacement of CB. Palm oil is an important source oil in the development of substitutes or equivalents for CB. The palm oil midfraction is very suitable substrate for CBEs production, because it is inexpensive and has an appropriate TAG composition [7]. The main component of PMF is the TAG POP, which can be converted to POS and SOS by enzymatic interesterification using an immobilized 1,3-specific lipase. The advantages of the enzymatic reaction over the chemical interesterification lie on its selectivity, mild reaction condition, few side reaction or by-products, ease to product recovery, easy process control, and minimal waste disposal [11].

The objective of this work was to study the enzymatic interesterification of palm oil midfraction and fully hydrogenated soybean oil using immobilized 1,3-specific lipase for the production of CBEs. This work also describes the effect of varying substrate weight ratios on the characteristics of the products in terms of their triacylglycerol compositions and of their melting profiles.

Materials and Methods

Materials

Palm oil midfraction (PMF) and cocoa butter (CB) were obtained from PT Karya Putrakreasi Nusantara, Wilmar Group, Medan, Indonesia. Fully hydrogenated soybean oil (FHSO) was a gift from Texas A&M University, USA. The lipase used was an immobilized preparation of a 1,3-specific lipase from *Thermomyces lanuginosa*, which is called Lipozyme TL IM

(Novozymes A/S, Bagsvaerd, Denmark). TAG standards were purchased from Sigma (St. Louis, MO USA).

Enzymatic Interesterification

PMF and FHSO were blended at weight percent ratios of 2:1 (A); 1.5:1 (B); 1:1 (C); 1:1.5 (D) and 1:2 (E). Solvent-free enzymatic interesterification of the blends was carried out in Erlenmeyer flasks (25 mL) with shaking (200 rpm) at 68-70°C within 4 hours. The reaction mixtures (5 g, total substrate mixture) were heated for 10 minutes at 68-70°C before addition of lipase (6 wt% of substrates). The product melt was quickly filtered through filter paper to remove the enzyme.

Fat Fractionation

A combination of fractionation steps of the interesterified product was performed using a modification of the procedures reported previously [5, 8]. First, the interesrterified product was neutralized to remove free fatty acids, and then it was purified by fractional crystallization in hexane and acetone. The interesterified product in hexane (1:5, wt%) was neutralized by 0.1 N NaOH (in 50% ethanol) with indicator of phenolphtalein. The upper phase was filtered through filter paper (+ Na_2SO_4) and then cooled at 4°C for 4 h. The precipitated solids were removed by filtration, and the filtrate was evaporated to dryness. The mother liquor was then dissolved in acetone (1:5, wt%) and cooled at 4°C for 4 h. The precipitated TAG crystlals were filtered at 4°C to give the CBEs.

TAG Composition

The TAG compositions of the starting blends, interesterified blends and the fat products (CBEs) were obtained by HPLC in a Hewlett Packard Series 1100 HPLC System equipped with a refractive index (RI) detector. A Zorbax Eclipse XDB C-18 (250 x 4.6 mm, Agilent Technologies Inc., USA) column in series with Microsorb MV (250 x 4.6 mm, Rainin Instrument Co. Inc., USA) column was used for the analysis with a mobile phase of 85:15 (v/v) acetone and acetonitrile at a flow rate of 0.8 mL/min. Each sample was dissolved in acetone or acetone/chloroform (2:1, v/v) to make a 5% solution. The injection volume was 20 μL. All TAG contents were given in percentage area.

Solid Fat Content (SFC)

The solid fat contents of the samples were measured using a Bruker Minispec PC 100 NMR Analyzer (Rheinstetten, Germany). The procedure was applied according to the IUPAC 2.150 (b) method (1987) [12]. The samples in the NMR tube were melted at 80°C and then held at 60°C for 30 min. The samples were then cooled at 0°C for 90 min. After cooling, the samples were kept at 26.5°C for 40 h, and then cooled at 0°C for 90 min. The samples were stabilized for 60 min at each measuring temperature (10, 20, 25, 30, 35 and 40°C) before measuring the liquid signal.

Slip Melting Point (SMP)

Slip melting point was determined according to AOCS Official Methods Cc 3-25, 2005[13]. SMP is the temperature at which a fat in a capillary tube placed in water becomes soft enough to slip or rise up the tube. The fats slip in the capillary tube when about 5% solid fat is present [14].

Results and Discussion

Enzymatic Interesterification

Table 1 shows the TAG composition of PMF, FHSO and their blends in various weight ratios. The main TAGs of PMF were POP (37.2%) and POO (18.7%), whereas the major TAGs of FHSO were PSS (42.5%) and SSS (26.5%). HPLC chromatograms of PMF and FHSO are shown in Figure 1. The TAG composition of fat blends represents a linear combination of the fat component in the blends. For example, as the proportion of PMF was increased in the blends, the proportion of POP, POO and other TAG present in PMF was increased.

Table 1. Triacylglycerol composition (area %) of the blends (PMF/FHSO) before and after interesterification at various weight ratios.

TAG (area%)	Substrate		Weight ratios of substrate (PMF/ FHSO)									
	PMF	FHSO	A (2:1)		B (1.5:1)		C (1:1)		D (1:1.5)		E (1:2)	
			BEI	AEI	BEI	AEI	BEI	AEI	BEI	AEI	BEI	AEI
PLL	1.8	-	1.3	0.7	1.5	0.6	1.3	0.5	1.2	0.3	1.1	0.4
OLO	1.6	-	1.3	1.3	1.3	1.0	1.2	0.6	1.2	0.4	1.2	-
PLO	8.1	-	5.9	4.1	5.4	3.2	4.7	2.5	3.8	1.8	3.7	1.4
PLP	8.6	-	5.8	2.8	5.3	2.9	4.4	1.8	3.6	1.4	3.0	1.1
OOO	3.3	-	2.7	1.9	3.0	1.3	2.7	0.8	2.5	0.5	2.4	-
POO	18.7	-	13.3	7.8	12.3	6.3	10.4	4.4	8.7	2.9	7.9	2.1
POP	37.2	-	25.0	11.0	22.2	9.5	18.5	7.3	14.8	5.4	12.8	4.5
PPP	1.6	1.3	1.5	4.4	1.5	4.2	1.5	3.6	1.3	2.9	1.3	2.7
SOO	2.3	-	1.9	4.8	1.7	4.7	1.6	4.2	1.3	3.5	1.2	2.9
POS	7.2	-	5.0	14.3	4.5	14.6	3.9	15.3	3.0	14.7	2.6	13.8
PPS	0.4	15.9	4.8	8.9	5.4	10.2	6.7	10.7	7.9	10.9	9.0	11.0
SOS	0.9	-	0.7	4.6	0.5	5.4	0.6	7.0	0.6	8.2	0.5	8.7
PSS	-	42.5	12.5	6.5	14.1	8.7	17.6	11.5	21.2	14.7	24.3	17.2
SSS	-	26.5	11.5	1.7	13.0	2.6	16.5	4.4	20.0	6.8	22.1	8.8
St3	2.0	86.2	30.3	21.5	34.0	25.8	42.3	30.3	50.5	35.3	56.7	40.5
St2U	53.9	-	36.4	32.7	32.6	32.4	27.2	31.5	21.9	29.8	18.9	28.1
StU2	30.9	-	22.3	8.8	20.9	7.9	18.0	6.7	15.1	5.3	13.8	4.3
U3	4.9	-	4.0	1.3	4.3	1.0	4.0	0.6	3.7	0.4	3.6	-

Abbreviations: BEI, before enzymatic interesterification; AEI, after enzymatic interesterification; L, linoleic acid; O, oleic acid; P, palmitic acid; S, stearic acid; St3, trisaturated; St2U, monounsaturated; StU2, monosaturated; U3, triunsaturated

Enzymatic interesterification of PMF and FHSO resulted in the formation of a complex mixture of acylglycerols and free fatty acids. Enzymatic interesterification also induced large changes in the TAG composition of the blends (Table 1). HPLC chromatograms of the blends before and after enzymatic interesterification are shown in Figure 1. The TAG profiles of the interesterified blends showed a more balanced or even peak distribution than the starting blends, as the relative concentration of several TAG increased, other decreased, and several new TAG might also have been synthesized. This result is consistent with findings reported by Noor Lida *et al.* [15] and Zainal and Yusoff [16].

Contents of target TAGs (POS, SOS) in the interesterified blends were increased, whereas the main TAGs of PMF (POP, POO) and TAGs of FHSO (PSS, SSS) were decreased. This result indicated that acyl exchange occured mainly between the palmitoyl group from the PMF and the stearoyl group from the FHSO. With the exception of blend E (PMF/FHSO 1:2, w/w), POS was the major TAG in the blends, with blend C having the highest amount of POS (Table 1). However, the highest content of SOS was observed in blend E.

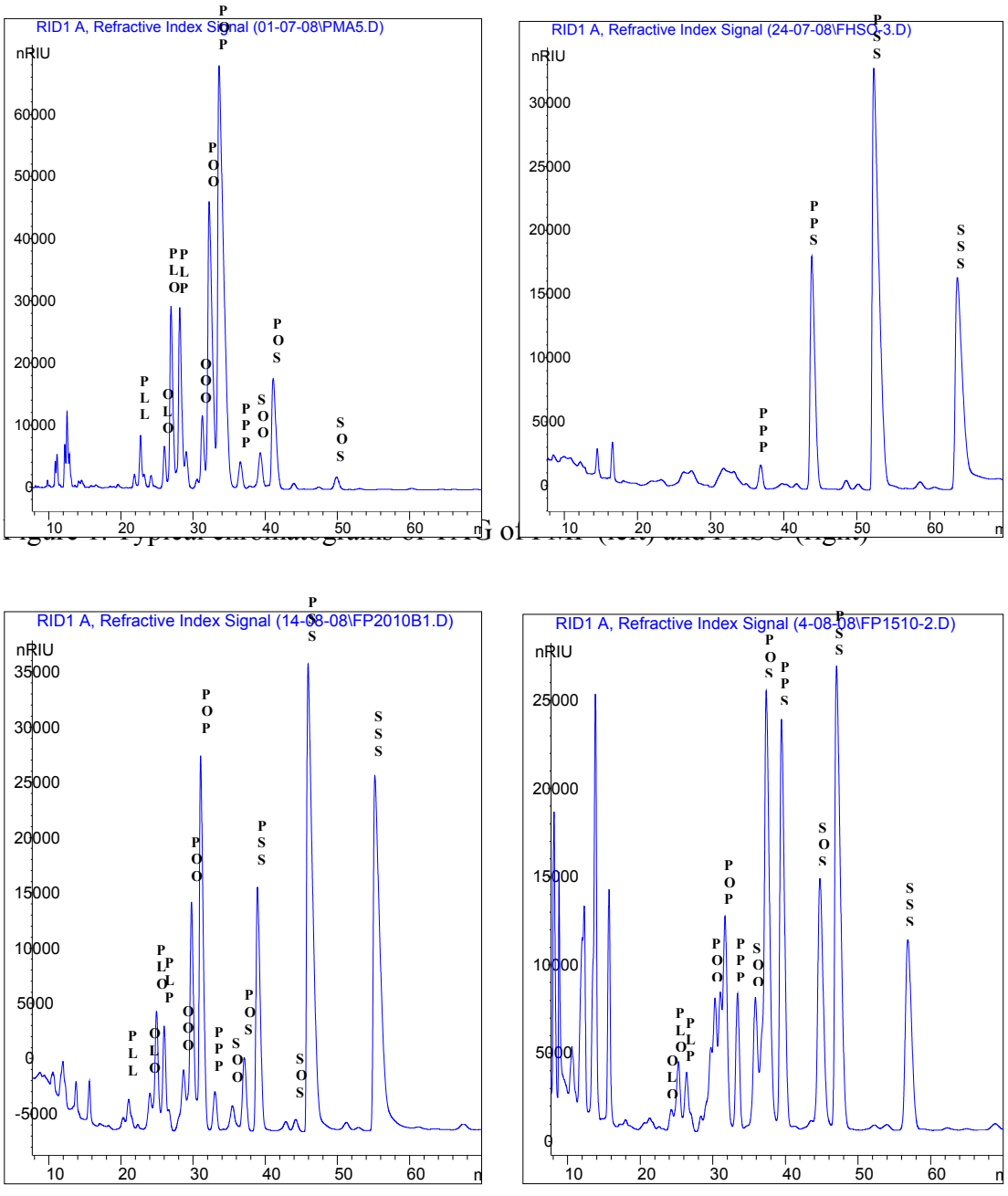

Figure 1. Typical chromatograms of TAG of the blends before (left) and after (right) enzymatic interesterification.

The resulting changes in the TAG composition of the blends were reflected in the SFC values. Figure 2 shows the SFC profiles of the blends before and after enzymatic interesterification. The SFC of PMF/FHSO blends did not represent linear combinations of the PMF and FHSO, this indicates the presence of some interaction between the components. A mixture with a eutectic effect will have a lower SFC than either one of the two pure fats, showing that the two fats are not compatible with each other [15]. SFC, i.e., the amount of fat crystals in the blends, is responsible for many product characteristics including general appearance, ease of packing, organoleptic properties, ease of spreading, and oil exudation [14].

Interesterified blends tended to have higher SFC values than the starting blends at low temperature (below 30-35°C) and lower SFC values at high temperature (above 30-35°C) with the exception of blend A and B at 10°C measuring temperature. This was due to the decrease in St3 TAGs (PPP, PPS, PSS, SSS) and StU2 TAGs (PLL, PLO, POO, SOO) also the increase in St2U TAGs (POP, POS, SOS). It appeared that the blends (before and after interesterified) containing high proportion of FHSO tended to have higher SFC values than the blends containing low proportion of FHSO at all measuring temperatures, which most probably was due to the content of high melting St3 TAGs and the content of low melting StU2 TAGs (higher or lower). The interesterified blends were solid at room temperature with SMP range of 45.2 to 50.3°C, which is lower than that of the starting blends (53.4 to 58.0°C). The decrease in SMP was induced mainly also by the changes in the TAG composition.

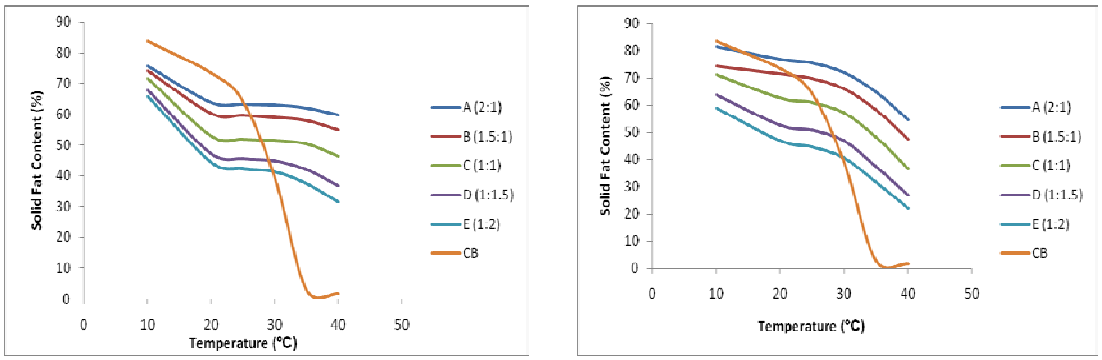

Figure 2. SFC values of the blends before (left) and after (right) enzymatic intereterification at various weight ratios of substrate (PMF/FHSO).

Fat Fractionation

Enzymatic interesterification of the PMF with FHSO resulted in the release of an equivalent amount of free fatty acids (FFA) and diacylglycerols (DAGs). Neutralized of the interesterified blends, followed by fractional crystallization of the fatty acid-free acylglycerols in hexane and acetone gave the fat products (CBEs) whose their TAG distribution comparable to that of CB but that they also contain DAGs (Table 2). HPLC chromatograms of the fat products (CBEs) and CB are shown in Figure 3. According to an EEC definition of CBE, all of the fat products can be termed as CBEs, because of the level of TAGs type StOSt ≥ 65% (St = saturated, O = oleic acid), except blend A. The increase in the content of targets TAGs (POS, SOS) of CBEs could be attributed mainly to the increased proportion of FHSO in the

blends. It appeared that the CBEs from the blends containing higher proportion of FHSO tended to have higher content of targets TAGs (POS, SOS).

The SFC profiles of the CBEs and CB are shown in Figure 4. SFC of the interesterified blends of PMF/FHSO were changed dramatically following fractional crystallization. The fat products (CBEs) tended to have lower SFC values than the interesterified blends, except at 10°C measuring temperature. This was due to the increase in St2U and StU2 TAGs and the decrease in St3 TAGs. Although the TAG distribution of CBEs comparable to that of CB, but the SFC profiles of CBEs less comparable to that of CB. The content of higher melting trisaturated (St3)TAGs, DAGs and FFA in the fat products (CBEs) has an adverse effect on melting properties [17]. Our products (CBEs) had SMP range of 30.0 to 34.5°C, compared to commercial CB with SMP of 31.8 to 32.6°C.

Table 2. Triacylglycerol composition (area %) of the fat products (CBEs) at various weight ratios of substrate (PMF/FHSO).

TAG (area %)	Weight ratios of substrate (PMF/FHSO)					Cocoa Butter
	A (2:1)	B (1.5:1)	C (1:1)	D (1:1.5)	E (1:2)	
OLO	1.3	1.1	0.6	0.4	0.3	0.8
PLO	3.4	3.0	2.0	1.5	1.2	1.7
PLP	3.4	2.9	2.3	1.9	1.6	0.5
OOO	2.0	1.6	0.9	0.6	0.4	2.2
POO	7.8	6.7	4.3	2.9	2.2	2.3
POP	**19.5**	**16.5**	**12.8**	**11.0**	**9.5**	**14.8**
PPP	1.3	1.2	1.5	1.2	1.4	0.9
SOO	5.5	5.7	4.8	4.1	3.5	2.9
POS	**30.1**	**32.6**	**35.1**	**37.5**	**37.7**	**36.8**
PPS	1.2	1.3	2.0	1.8	2.0	0.7
SOS	**9.7**	**13.3**	**18.5**	**22.9**	**26.0**	**25.3**
PSS	0.4	0.5	1.1	0.9	1.0	0.7
SOA	0.5	0.5	0.7	0.7	0.8	1.5
DAG	5.2	4.4	4.9	3.7	3.4	2.4
Others	8.7	8.7	8.5	8.9	9.0	5.6
St3	2.9	3.0	4.6	3.9	4.4	2.3
St2U	63.2	65.8	69.2	74.0	75.5	78.9
StU2	16.3	15.4	11.1	8.6	6.9	13.8
U3	3.3	2.9	1.5	0.4	0.3	3.0

Abbreviations : L, linoleic acid; O, oleic acid; P, palmitic acid; S, stearic acid; DAG, diacylglycerol; Others, unidentified TAG. For other abbreviation see Table 1.

The substrate weight ratio of PMF to FHSO to produce a fat product (CBE) containing the highest major TAG component of CB (POS, 37.7% and SOS, 26.0%) was 1:2 (blend E). However, our product (CBE) had a lower POP content (9.5%) than that of commercial CB (14.8%). The commercial CB used in this study contained 14.8% POP, 36.8% POS and 25.3% SOS. The SMP of 31.8-33.4°C observed for blend E compares well to the melting point of CB-like fats as measured by DSC as reported by other authors: Ciftci *et al.* [4], 29.9°C;

Abigor *et al.* [5], 33.8°C; Liu *et al.* [6], 34.3°C; Chang *et al.* [9], 39°C. We can also conclude that the most similar SFC profile to CB was obtained from blend E (Figure 4).

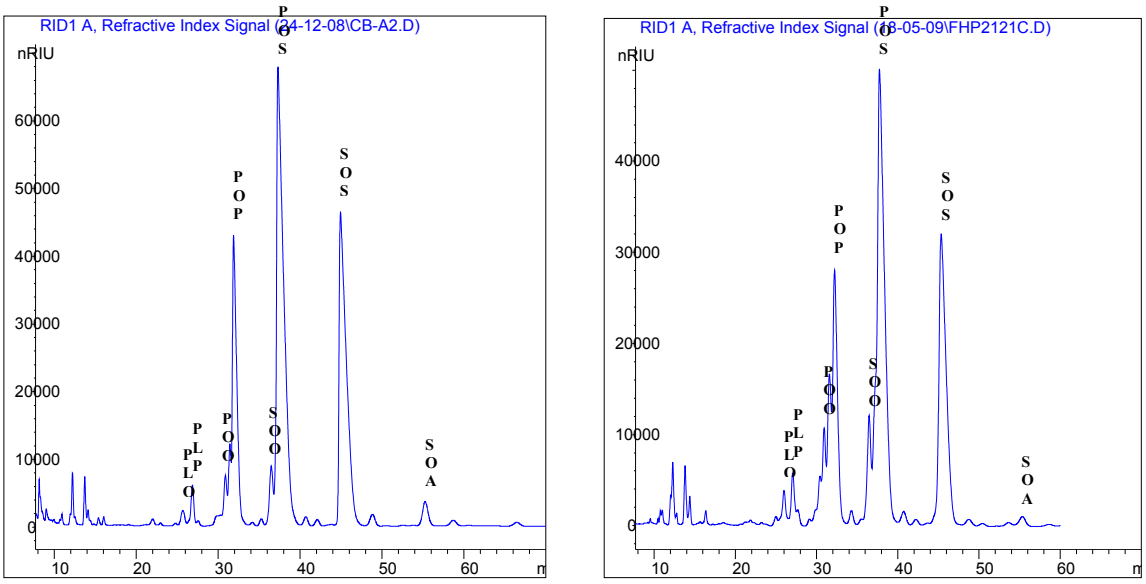

Figure 3. Typical chromatograms of TAG of CB (left) and the CBEs (right).

The highest yield of a fat product (CBE) was about 20.5% based on the weight of the original substrate. Other authors have achieved different yields of CBEs. Chang *et al.* [9] reported a yield of CB-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 [6]. The yield of CB-like fat of 45.6% also reported by Abigor *et al.* [5] when refined, bleached, deodorized palm oil (RBDPO) was interesterified with fully hydrogenated soybean oil (FHSO) at a weight ratio of 1.6:1.

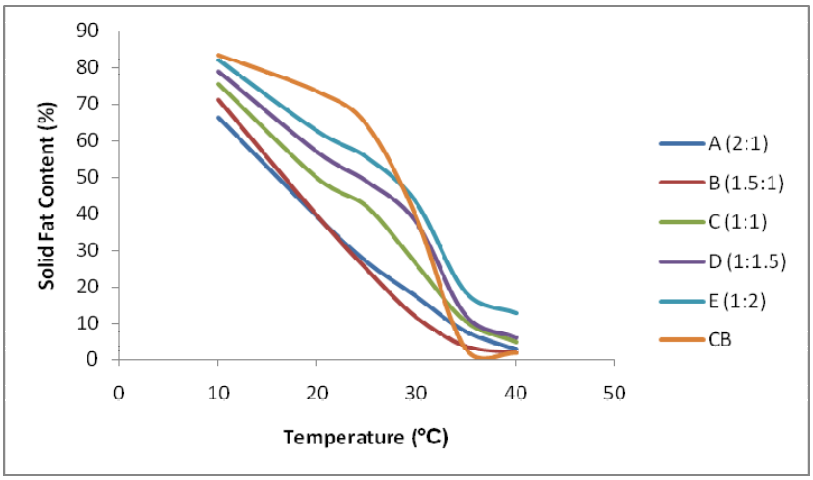

Figure 4. SFC values of CBEs at various weight ratios of substrate (PMF/FHSO).

Conclusion

The highest major TAGs component of CB (9.5% POP, 37.7% POS, 26.0% SOS) in CBE was obtained at 1:2 substrate (PMF/FHSO) weight ratio, 6% enzyme load, 4 h reaction time, 68-70°C reaction temperature, and 200 rpm speed of orbital shaker. Although the content of POP TAG in our CBE was very low, we can conclude that PMF is a suitable source for CBEs production using Lipozyme TL IM, sn-1,3 specific lipase.

Acknowledgment

Thanks are addressed to the Ministry of Research and Technology the Republic of Indonesia for funding this research through National Strategic Prime Research of Oil Palm Industry (RUSNAS) program.

References

1. Gunstone, F.D. (2002). Food Applications of Lipids. In C.C. Akoh, and D.B. Min (eds). Food Lipids Chemisty, Nutrition, and Biotechnology. Second Edition, Revised and Expanded. Marcel Dekker, Inc., New York.

2. Wainwright, R.E. (1996). Oils and Fats in Confections. In Y.H. Hui (ed). Bailey's Industrial Oil and Fat Products. Fifth Edition Volume 3 Edible Oil and Fat Products: Products and Application Technology. John Wiley & Sons, Inc., New York.

3. Torbica, A., O. Jovanovic, and B. Pajin. (2006). The advantages of solid fat content determination in cocoa butter and cocoa butter equivalents by the Karlshamns method. **European Food Research and Technology**, 222:385-391.

4. Ciftci, O.N., S. Fadiloglu, and F. Gogus. (2009). Utilization of Olive-Pomace Oil for Enzymatic Production of Cocoa Butter-like Fat. **Journal of the American Oil Chemists Society**, 86:119-125.

5. Abigor, R.D., W.N. Marmer, T.A. Foglia, K.C. Jones, R.J. DiCiccio, R. Ashby, and P.O. Uadia. (2003). Production of Cocoa Butter-like Fats by the Lipase-Catalyzed Interesterification of Palm Oil and Hydrogenated Soybean Oil. **J. Am. Oil Chem. Soc.** 80(12):1193-1196.

6. Liu, K.J., H.M. Cheng, R.C. Chang, and J.F. Shaw (1997). Synthesis of Cocoa Butter Equivalent by Lipase-Catalyzed Interesterification in Supercritical Carbon Dioxide. **J. Am. Oil Chem. Soc.** 74(11):1477-1482.

7. Mojovic, L., S.S. Marinkovic, G. Kukic, and G.V. Novakovic (1993). *Rhizopus arrhizus* Lipase-catalyzed Interesterification of the Midfraction of Palm Oil to a Cocoa Butter Equivalent Fat. **Enzyme and Microbial Technology**, 15:438-443.

8. Chong, C.N., Y.M. Hoh, and C.W. Wang (1992). Fractionation Procedures for Obtaining Cocoa Butter-like Fat from Enzymatically Interesterified Palm Olein. **J. Am. Oil Chem. Soc.** 69(2):137-140.

9. Chang, M.K., G. Abraham, and V.T. John (1990). Production of Cocoa Butter-Like Fat from Interesterification of Vegetable Oils. **J. Am. Oil Chem. Soc.** 67(11):832-834.

10. Idris, N.A., and N.L.H.M. Dian (2005). Interesterified Palm Products as Alternatives to Hydrogenation. **Asia Pacific Journal of Clinical Nutrition**, 14(4):396-401.

11. Yang, T., M.B. Fruekilde, and X. Xu (2003). Applications of Immobilized *Thermomyces lanuginosa* Lipase in Interesterification. **JAOCS** 80(9):881-887.

12. IUPAC NORM VERSION. 1987. 2.150 (ex 2.323). Solid Content Determination in Fats by NMR (Low Resolution Nuclear Magnetic Resonance).

13. American Oil Chemists' Society (2005). Official Methods and Recommended Practices of the American Chemists' Society. Am. Oil Chem. Soc., Champaign, Illinois.

14. Noor Lida, H.M.D. and A. Rahim Md. Ali (1998). Physicochemical Characteristics of Palm-Based Oil Blends for the Production Of Reduced Fat Spreads. **J. Am. Oil Chem. Soc.** 75(11):1625-1631.

15. Noor Lida, H.M.D., K. Sundram, W.L. Siew, A. Aminah, and S. Mamot (2002). TAG Composition and Solid Fat Content of Palm Oil, Sunflower Oil, and Palm Kernel Olein Blends Before and After Chemical Interesterification. **J. Am. Oil Chem. Soc.** 79(11):1137-1144.

16. Zainal, Z. and M.S.A. Yusoff (1999). Enzymatic Interesterification of Palm Stearin and Palm Kernel Olein. **J. Am. Oil Chem. Soc.** 76:1003-1008.

17. Hashimoto, S., T. Nezu, H. Arakawa, T. Ito and S. Maruzeni (2001). Preparation of Sharp-Melting Hard Palm Midfraction and Its Use as Hard Butter in Chocolate. **J. Am. Oil Chem. Soc.** 78(5):455-460.