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Enzymatic Interesterification of Palm Products for Producing Low Calorie Cocoa Butter Substitutes

[1]Ros-Haniza Borhan, [2]Mamot Said and [2]Miskandar Mat Sahri
[1]Food Science Programme, School of Chemistry Science and Food Technology,
Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
[2]Malaysian Palm Oil Board, P.O. Box 10620, 50720, Kuala Lumpur, Malaysia

Abstract: An immobilized sn-1, 3-specific lipase from Rhizomucor miehei was used to catalyze the interesterification of Palm Mid Fraction (PMF), Palm Kernel Stearin (PKS) and Medium Chain Triacylglycerols (MCT) under controlled reaction parameters such as enzyme load, time course and temperature to produce a low calorie Cocoa Butter Substitutes (CBS). Samples were analyzed for physical (slip melting point, solid fat content) and chemical properties (fatty acids and triacylglycerol composition) and compared with cocoa butter. Quantitative analysis of the reaction mixture was conducted by using High Performance Liquid Chromatography (HPLC), which enabled the exact tracing of the triacylglycerol synthesis of rearrangement. In this study, 4 samples based on palm products (PMF, PKS, PMF: PKS, EIE PMF: PKS: MCT) were analysed including cocoa butter. The solid fat content for all samples at all measured temperature showed the sharp melting properties in a range of 15° to 30°C. A-PMF showed higher SMP (34°C) compared to others and the lowest was D-EIE fat (31°C) which means that the structured CBS is softer. The rearrangement of fatty acids between medium chain fatty acids and long chain fatty acids contribute structured triacylglycerols which might affected the calorie value of the CBS.

Key words: Medium chain triglycerides, solid fat content, melting point, enzyme RM IM, triacylglycerol

INTRODUCTION

The uncertainty in supply and the volatility in prices of cocoa butter have led confectioners to seek economical vegetable fats to replace cocoa butter in chocolate and confectionery products. Cocoa Butter (CB) is currently the fat of choice in the confectionery industry. Its polymorphism greatly affects the physical properties of chocolate products, such as gloss, snap, contraction, heat resistance, quick and sharp melting in the mouth and bloom-resistance (Loisel *et al*., 1998; Osborn and Akoh, 2002). Cocoa butter contains more than 50% saturated fatty acids (SFAs). A CBS is a fat that provides some of the desired physical characteristics to a confection independent of its dissimilar chemical composition to that of cocoa butter (Lawler and Dimick, 1998). The use of CBS recently is more common as the cost for CB has increased.

Palm Mid Fraction (PMF), the mid fraction from the double fractionation of palm olein is rich in POP (1,3-dipalmitoil-2-oleoil-sn-gliserol). Fats rich in POP triacylglycerols and closely resembling cocoa butter are used as confectionery fats throughout the world for cost savings (Anon, 1994). On the other hand, Refined, Bleached and Deodorized Palm Kernel Stearin (RBD PKS) is used for high quality confectionery fats (Wiedeman, 1991).

Interesterification (IE) is a powerful tool for modifying the physical and chemical properties of oils and fats. IE involves redistribution and interchange of fatty acids (FAs) within and between the triaclglycerol (TAG) molecules, which make up all oils and fats (Xu, 2000). The result is a significantly changed TAG composition and consequently altered melting and crystallization behaviours of the oils and fats. IE does not result in the formation of either trans or geometrical isomers of FAs. Partially hydrogenated fats can be tailored for specific applications. However, they contain trans FAs because during the process of hydrogenation, some of the FA double bonds are isomerized into trans FAs from their *cis* configuration (Ghotra *et al*., 2002). Trans FAs have been proven to raise low-density lipoprotein (bad) cholesterol levels, causing the arteries to become more rigid and clogged and increase the risk for cardiovascular disease (Yella and Jeyarani, 2001).

Long-chain Triacylglycerol (LCT) emulsions alone are not effective as energy sources because they are

Corresponding Author: Ros-Haniza Borhan, Food Science Programme, School of Chemistry Science and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

metabolized slowly compared to Medium-Chain Triacylglycerols (MCT). Because Long-Chain Fatty Acids (LCFA) are transported through the lymphatic system by incorporation into chylomicrons, it takes longer for them to get to the liver and supply the needed energy. MCT or Medium-Chain Fatty Acids (MCFA) have been introduced into LCT as a source of quick energy from fat (Akoh and Yee, 1997). MCT are unique in that they are not carnitine-dependent for transport across the mitochondria are not incorporated into chylomicrons and are not readily stored in adipose tissue.

Low calorie cocoa butter substitutes can be produced from mixtures of MCT and LCT by enzymatic interesterification. The use of enzymes is a viable means of producing SL because specific fatty acids at specific positions of the glycerol moiety can be produced, delivering lipids with maximum nutritional and functional properties. Another method is through physical blending of individual MCT and LCT. Use of a low-calorie lipid is of interest. SL with saturated medium-chain fatty acids can be intentionally produced because they provide about 3.5-6 kcal g^{-1} whereas, long-chain fatty acids provide about 9-9.5 kcal g^{-1} (Lee *et al*., 2008). Although the energy and nutritional requirements are met, the original absorption rates of individual TAG are retained, and physical mixtures are therefore not equivalent to SL. Consequently, patients with malabsorption conditions cannot effectively use them.

The objective of this research was to produce low-calorie cocoa butter substitutes with specific fatty acids at the *sn*-1, 3 positions by interesterifying MCT, palm mid fraction and palm kernel stearin with *sn*-1,3-specific immobilized lipase IM 60 from *Rhizomucor miehei*.

MATERIALS AND METHODS

Materials: Palm Mid Fraction (PMF), Palm Kernel Stearin (PKS) and Medium Chain Triacylglycerol (MCT) were supplied by Malaysian Palm Oil Board (Bangi, Malaysia). Immobilized *sn*-1,3-specific lipase IM 60 from *R. miehei* was obtained from Novo Nordisk Biochem North America Inc. (Franklinton, NC).

Solid fat content analysis (SFC): Solid Fat Content (SFC) was determined using a Bruker Minispec PC 120 Pulse Nuclear Magnetic Resonance (p-NMR) analyser (Karlsruhe, Germany) according to the procedure described in MPOB (2005). The sample in the pNMR tube was first melted at 70°C for 30 min, followed by chilling at 0°C for 90 min. It was then held at each measuring temperature for 30 min before measurement. SFC was measured in the temperature range of 5°-35°C.

Slip melting point (SMP): SMP was determined according to MPOB (2005).

Triacylglycerols (TAG): The TG profiles were analysed in a reversed-phase high-performance liquid chromatography. A Lichrosphere RP-18 column (250 mm) of 5 μm particle size (Merck, Darmstadt, Germany) with acetone/acetonitrile (75:25% v/v) as the eluent at a flow rate of 1.0 mL min^{-1} and a refractive index detector was used. Identification of TG was done by comparison of retention time with those of commercial TG standards.

Fatty Acid Composition (FAC): Fatty acid methyl esters were prepared as described in MPOB (2005).

RESULTS AND DISCUSSION

Slip melting point: SMP related with temperature whereby it can determine the crystallisation and melting behaviour. The effect depends on the changes of polymorph form. Palm kernel based has wide range of melting point. Owing to its unique characteristics, palm kernel oil is suitable for producing specialty fats mainly for confectionery use. Palm kernel oil was classified as lauric fat as it contains a high percentage of lauric acid (48%). Because of its high content of oleic acid, it is suitable for hydrogenation and interesterification process in the production of specialty fats (Goh, 1994). By controlling the process, a series of palm kernel based products with melting points ranging from 32 to 41°C can be produced to suit various applications. Samples exhibited SMPs below body temperature within range 31°C to 34°C. The SMP of the interesterified fat, palm fats and blend are shown in the Table 1. The interesterified blends had lower SMP than other fats analysed. The changed of SMP was influenced by both short chain and unsaturated fatty acids (Siew and Berger, 1981). The higher SMP was due to high amount of high-melting TGs, such as POP and PPP, in PMF compared to PKS. Norizzah *et al*. (2004) evaluated melting thermograms of interesterified palm products and confirmed a product of lower melting point being formed in interesterified blends with the disappearance of the high melting glycerides.

Table 1: Slip melting point

Sample	SMP
A	34.0[a]
B	33.1[a]
C	32.0[a]
D	31.0[a]
E	33.0[a]

Mean values followed by similar superscripts within the same column are not significantly different (p>0.05) A: Palm Mid Fraction (PMF), B: Palm Kernel Stearin (PKS), C: Blends of PMF:PKS, D: Interesterified blends dan E: Cocoa Butter

Solid fat content: Solid fat content for different fats or blends can be influenced by fatty acids and triacylglycerol obtained through process approached. Bigalli (1988) reported that when two substances are melted together, the blend may have the tendency to solidify at a lower temperature than either one of its constituents a behavior known as eutectic effect. Referring to the SFC results, it was found that interesterification had lowered the percentage of SFC of each blend at each temperature measurement and also lowered the slip melting point of each blend. PMF and PKS displayed sharp solid fat content profiles with relatively high solids at 25°C and below while at 35°C the fats were completely melted. Assuming there were not expected to contribute any waxy taste to the confectionery products due to the both fats melted completely at 35°C (Sabariah and Rahim, 1996). Pease (1985) concluded that samples formulated from lauric cocoa butter substitute exhibited good mouthfeel and rich flavor release owing to the narrow melting range. Figure 1 showed the SFC profiles of the palm fats, interesterified blends and cocoa butter as a function of temperature, respectively. Changes of solid fat content at specific temperature range are related with some characteristic such as hardness, heat resistant at 25° to 30°C and melting properties at 27° to 33°C. The interesterified fat showed lower SFC at all measured temperatures meant that it was softer than others. After interesterification, the steepness of decrease in most blends had decreased for the same temperature range. Flattening effect was most probably caused by the existence of a wider variation of triacylglycerols as a result of interesterification (Timms, 1979). This could be due to the rearrangement of fatty acids in the TGs after interesterification, to form a medium melting fraction and a low melting fraction. Slight changes in the melting point of fats had a distinctive effect on the SFC (Laning, 1985).

Triacylglycerols (TAG): Structured lipids (SLs) are tailor-made fats and oils with improved nutritional or physical properties because of modifications such as enzymatic interesterification process to incorporate new fatty acids or to change the position. SL synthesis yields novel triacylglycerol (TAG) molecules (Akoh, 1998). SLs may provide the most effective means of delivering desired fatty acids for nutritive or therapeutic purposes and for targeting specific diseases and metabolic conditions (Lee *et al*., 2008) of existing fatty acids on the glycerol backbone. The structured triacylglycerol was due to random rearrangement of the fatty acids during interesterification. Reduced calorie SLs are designed by taking advantage of either limited absorption of long-chain saturates or the low caloric value of short chain fatty acids (Osborn and Akoh, 2002). Changes in the TAG profiles are usually accompanied by changes in the physical characteristics, such as SMP and SFC of the oil blends. Since the concentrations of the high melting TAG, such as PPP and POP, were reduced after interesterification, a softer product was obtained. Table 2 showed the major type of triacylglycerols for all samples tested. The higher percentage of short and medium chain fatty acids in PKS (70.1%) was reflected in the higher percentage of triacylglycerols with lower carbon number compared to PMF that consist 84.8% of POP. Sample D showed the combination of MCT and POP triacylglycerols which may affect the calorie value of the final products.

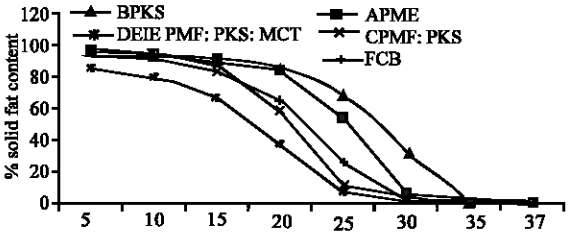

Fig. 1: Solid fat content of Palm Mid Fraction (PMF), Palm Kernel Stearin (PKS), Blends of PMF:PKS, Interesterified blends dan and Cocoa Butter

Table 2: Triacylglycerols composition

Sample	MCT	POP	PPP	POS	SOS
A		84.8	0.7	8.4	
0	70.1	0.10			
C	19.4	58.2	0.8	8.9	
D	27.1	57.1	0.4	6.6	
E		17.0		51.4	28.2

A: Palm Mid Fraction (PMF), B: Palm Kernel Stearin (PKS), C: Blends of PMF:PKS, D: Interesterified blends dan E: Cocoa Butter

Fatty acid composition (FAC): Palm products have high content of palmitic acid and oleic acids. Palmitic acid, especially at the sn-2 position, has been associated with increases in atherogenicity (Kritchevsky *et al*., 1998). Stearic and oleic acids are neutral with respect to serum cholesterol (Decker, 1996). Table 3 showed PKS consisted high amount of short and medium chain saturated fatty acids, C8:0 to C14:0 and contained low amounts of unsaturated fatty acids, C18:1 and C18:2. According to Wong (1991), PKS with 55.4% C12:0 and 21.8% C14:0 could be used to produce a sharp melting CBS at body temperature. On the other hand, PMF contained more unsaturated fatty acids (33%) and C16:0 about 57%. Sample D displayed the balancing of medium chain fatty acids and long chain fatty acids purposely to produce the low calorie Cocoa Butter Substitutes (CBS)

Table 3: Fatty acids composition

Sample	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0	22:0
A				0.8	57.4	6.0	31.9	0.3	0.4	3.2	
B	1.9	2.8	55.4	21.8	8.50	1.9	6.40			0.9	
C	0.5	0.7	13.1	5.8	45.8	5.1	26.3	2.7			
D	7.2	5.3	12.1	5.4	40.0	4.3	23.0	2.3			
E				0.1	25.9	36.5	32.7	0.3	1.2	2.4	0.2

A: Palm Mid Fraction (PMF), B: Palm Kernel Stearin (PKS), C: Blends of PMF:PKS, D: interesterified blends dan E: Cocoa Butter

compared to CB that only containing long chain fatty acids. Cocoa Butter (CB) contains approximately 26% palmitic acid, 37% stearic acid and 33% oleic acid. These values are similar to those previously reported (Chapman *et al*., 1971; Grompone, 1984; Nesaretnam and Ali, 1992; Zheng and Hanna, 1996; Bayard and Wolff, 1996). However, enzymatic modification was unable to produce lipids with levels of oleic acid similar to CB at the sn-2 position.

CONCLUSION

Enzymatic interesterification was an effective process to make rearrangement of lipid structure. The EIE process obtained the low Calorie Cocoa Butter Substitutes (CBS) that contained triacylglycerol with a combination of medium chain fatty acids and long chain fatty acids. While for the SFC showed similar trend of curve and melting point did not showed any significant different ($p>0.05$) between palm fat and cocoa butter. The specifications of interesterified fat are comparable with cocoa butter but showed slightly softer.

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