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Full Length Research Paper

High lauric human milk fat analog prepared from palm stearin and coconut oil by enzymatic interesterification

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In this study, an attempt to synthesize palm and coconut based Human Milk Fat analog (HMF analog) with high percentage of palmitic acid in the sn-2 position and high lauric acid in the sn-1,3 positions of the triglycerides was carried out. The first step was preparation of monoglycerides by hydrolysis of palm stearin, which is rich in palmitic and oleic acids in their sn-2, using porcine pancreatic lipase, which has specificity in sn-1,3. The 2-monoglycerides (2-MG) product were separated and purified by thin layer chromatography (TLC), and it was confirmed having 55.21% of palmitic acid and 35.26% of oleic acid. The second step was interesterification of the 2-MG from palm stearin with high lauric fatty acid methyl ester, previously obtained by methanolysis of coconut oil, to synthesize HMF analog using immobilized lipase from *Rhizomucor miehei* (lipozyme RM IM) as biocatalyst. Enzymatic interesterification reactions were held at various reaction temperatures (50, 55 and 60°C), reaction times (6, 12, 18 and 24 h) and enzyme concentrations (2.5, 5.0, 7.5 and 10.0 wt% of total substrate). The interesterification condition was temperature of 50°C for 12 h of reaction time and enzyme concentration of 10.0% (w/w). The content of Medium Chain Fatty Acids (MCFA) in the HMF analog was 43.86% and the main constituent was lauric acid (39.37%), whereas the sn-2 position was dominated by palmitic acid (24.18%).

Key words: Coconut oil, palm stearin, human milk fat analog, high lauric, enzymatic interesterification.

INTRODUCTION

Human milk is a naturally the only source of food for infants in their early life. However, for some reasons many mothers have to depend on infant milk formulas to feed their babies. A significant differences between human milk fat (HMF) and infant milk fat formulas is positional distribution of acyl groups bonded to the glycerol. The distinct characteristic of human milk fat is the high percentage of palmitic acid (18.62 to 23.02%), that predominantly located in the sn-2 position of the triglycerides (Yuhas et al., 2006), meanwhile infant formulas contain palmitic acid predominantly in sn-1,3 positions (Schmid et al., 1998). The role of palmitic acid in the sn-2 position of the glycerol backbone is to ease the digestion and absorption of the fats in the infant intestine. Long chain saturated fatty acids, like palmitic acid, esterified to sn-1,3 positions during the digestion can form insoluble fatty acid complexes with calcium rendering it unavailable (Carnielli et al., 1998).

Lauric acid, as a member of Medium Chain Fatty Acids (MCFA), which in the Virgin Coconut Oil (VCO) amounted 46.64 to 48.03% (Marina et al., 2009), was found to increase body endogenous oxidation by changing the composition of the adipose tissue pool through altered endogenous availability. The capability of Medium Chain Triglyceride (MCT) to increase endogenous fat oxidation

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could have implications in the reduction of adipose tissue mass by increasing adipose tissue mobilization (Binnert et al., 1998; Papamandjaris et al., 2000).

This research attempted to synthesize human milk fat analog having high percentage of palmitic acid in the sn-2 and high lauric acid in the sn-1,3 positions through enzymatic interesterification. Enzymatic interesterification was a method to synthesis triacylglycerol by direct esterification of ester with other ester. Other methods to restructure a new triacylglycerol can be take place through direct esterification of ester and acid (acidoysis), or through reactions between ester with alcohol (alcoholysis) (Gunstone, 1999; Willis and Marangoni, 2002). Lipases were the enzyme used to restructure the triacylglycerol. These enzyme not only serve the hydrolysis process, but are also capable of catalysing the reverse reaction to produce esters under certain conditions. The hydrolysis occurs in aqueous media when there is large of water, meanwhile the esterification achieves under low water condition (Divakar and Manohar, 2007). In the present research, we used 2 kinds of lipases namely porcine pancreatic lipase and an immobilized lipase from *Rhizomucor miehei*. Mendes et al. (2012) reported that porcine pancreatic lipase has high stability and showed capability to hydrolyze the sn-1,3 position of fatty acid in the triglycerides. Lipase from *R. miehei* has been used widely as biocatalyst in fats and oils modification (Rodrigues and Fernandez-Lafuante, 2010). Concerning the capability of these two lipases to catalyse the reaction, we used the porcine pancreatic lipase to hydrolize palm stearin and an immobilized lipase from *R. miehei* to synthesis human milk fat analog.

To our knowledge, up to date, there was no available data regarding the synthesis of human milk fat analog from high palmitate 2-monoglycerides and high lauric fatty acid methyl ester. In the present work, we report: a) hydrolysis of palm stearin with porcine pancreatic lipase to obtain high palmitate 2-monoglycerides; and b) synthesis of the separated 2-monoglycerides with high lauric fatty acid methyl ester. The effect of reaction temperature, incubation time, and enzyme concentration during interesterification of 2-monopalmitin with high lauric fatty acid methyl ester were investigated.

MATERIALS AND METHODS

Palm stearin was purchased from local palm oil plant in Bitung, North Sulawesi, Indonesia. VCO was extracted from fruit of Mapanget Tall Coconut variety (11 to 12 months) which was obtained from Kima Atas experimental garden Manado. An immobilized Lipozyme RM IM from *R. miehei*, Porcine pancreatic lipase type II, and lipid standards such as trilaurin, lauric acid, and 1,3 dipalmitin were purchased from Sigma-Aldrich, Netherlands. Standard 2-monopalmitin was obtained from Larodan, Sweden. Silica gel F254 plates (20 × 20 cm) was purchased from Merck, Germany. All solvents were of analytical grade and were purchased from Merck, Germany.

Preparation of fatty acid methyl ester from coconut oil

Potassium metoxide solution was prepared by dissolving 4.2 g of potassium hydroxide in 97 ml of methanol. This solution was added into 300 g of VCO in 500 ml glass round vessel equipped with condensor and a thermometer. Methanolysis reaction was carried out at 50°C for 2 h on a hot plate. The reaction products were then transferred into a separating funnel and allowed to separate for 2 h. The upper layer, which is fatty acid methyl ester was separated from the remaining glycerol in the lower layer. The fatty acid methyl ester was transferred into a clean separating funnel and washed 3 times with 500 ml of water while the glycerol was discarded. The fatty acid methyl ester layer was then passed through anhydrous sodium sulfate 3% (w/v, of fatty acid methyl ester) to remove any residual water. The fatty acids methyl ester were then determined by Gas Chromatography.

Hydrolysis of palm stearin to obtain high palmitate 2-monoglyceride

The condition was optimized in our previous study (Karouw et al., 2012). The process was conducted as follow: A reaction mixture was prepared consisted of 2 g palm stearin, 8 ml isooctane, and 800 ml phospate buffer pH 6.5. Porcine pancreatic lipase Type II was added into the reaction mixture. The amount of porcine pancreatic lipase was 5% (w/w) of weight of palm stearin in the reaction mixture. Enzymatic hydrolysis reaction was carried out at temperature of 37°C for 42 h in a waterbath shaker with speed of 80 stroke/min. At the end of the reaction, lipase was separated from reaction mixture by centrifugation, and the solvents were removed under a stream of N_2 gas. Leaving reaction products at the bottom of the flask.

Separation of 2-monoglyceride

The 2-monoglyceride was isolated from the reaction products by thin layer chromatography (TLC). The reaction products were dissolved in chloroform and then applied to TLC plates, developed in petroleum ether:diethyl ether:acetic acid (60:40:1, vol/vol/vol) solvent. The separated spots were visuallized by holding the plate in iodine vapor. The bands corresponding to 2-monoacylglycerol (2-MAG) were scrapped off and extracted 2 times with 5 ml of diethyl ether. The extract was collected into a test tube and the solvents was removed under a stream of N_2 gas. The amount of purified 2-monoglyceride was estimated from the difference between the empty test tube weight and that with sample.

Interesterification reaction

The purified 2-monoglycerides in the test tube were mixed with fatty acid methyl ester (3 times of 2-monoglyceride estimated weight) and dissolved in 3 ml of hexane. Lipozyme RM IM enzyme (10%) was added into the mixture to optimize the enzymatic interesterification temperature (50, 55 and 60^{o}C) and time (6, 12, 18, 24 h). These two experiments produced optimum time and temperature, which then to be used to determine optimum enzyme concentration. Lipase RM IM of 2.5, 5.0, 7.5, and 10.0 wt% of total substrate was used to find the optimum concentration operating under the optimum temperature and time previously determined. All of the enzymatic interesterification reactions were carried out in a waterbath shaker operating at 120 stroke/min. At the end of the experimental reaction, lipase was separated from the mixture by centrifugation. Solvents in the supernatant containing the triglycerides was evaporated under N_2 gas flux and the remaining sediment was used for further triglyceride composition analysis.

Table 1. Fatty acid composition (%) of palm stearin, coconut oil and fatty acid methyl esters of coconut oil.

Fatty acids	Palm stearin[a]	Coconut oil[b]	FAME of coconut oil[c]
C8:0	ND	7.41 ± 0.38	5.95 ± 0.13
C10:0	ND	6.28 ± 0.14	6.02 ± 0.04
C12:0	ND	48.24 ± 0.07	48.84 ± 1.33
C14:0	ND	19.26 ± 0.09	19.36 ± 0.13
C16:0	59.41 ± 1.10	9.29 ± 0.02	9.48 ± 0.86
C18:0	4.99 ± 1.16	2.44 ± 0.06	2.35 ± 0.24
C18:1	29.59 ± 0.05	5.83 ± 0.30	6.51 ± 0.76
C18:2	6.00 ± 0.11	1.26 ± 0.10	1.42 ± 0.25

ND, not detected; FAME, fatty acids methyl ester. [a]Purchased from palm oil plant located in Bitung, North Sulawesi, Indonesia. [b]Extracted from 11 to 12 months fruit of Mapanget Tall coconut which grown in Kima Atas experimental garden belongs to Indonesia Palmae Research Institute, North Sulawesi, Indonesia. [c]Synthesis by chemical methanolysis of coconut oil with potassium hydroxide as biocatalyst at 50°C for 2 h.

Glycerides composition analysis

This step consists of triglyceride separation, purification, preparation of fatty acid methyl esters, and fatty acid identification by Gas Chromatography as follows:

Triglycerides separation

The sediment obtained from interesterification reaction was dissolved in chloroform (0.1 g of sediment/ml of chlorofom) and then 20 □l of the solution was applied to TLC plates and developed in petroleum ether:diethyl ether:acetic acid (60:40:1, vol/vol/vol). Spots were then analyzed using Camag TLC-scanner. The relative percentage of monoglycerides (MG), diglycerides (DG), triglycerides (TG), and free fatty acids (FFA) were based on the total area of the spots.

Triglycerides purification

The bands were visualized by holding the plate in iodine vapor. The bands corresponding to triacylgycerol (TAG) (having Rf corresponding to TAG standard) were scrapped off and extracted twice with diethyl ether. The solvents were removed under a stream of N_2 gas flux. The fatty acid profile of the TAG were analyzed by Gas Chromatography (GC).

Preparation of fatty acid methyl esters

Fatty acid methyl esters of isolated TAG were prepared by transesterification in the presence of methanol and potassium chloride. A solution of TAG-hexane 5% was prepared by dissolving isolated TAG in hexane. Into the 6 ml of TAG-hexane solution, 150 ml of 2.0 N potassium hydroxide was added. The mixture was then homogenized for 5 min and centrifuged for 5 min at 3000 rpm. The fatty acid methyl esters in supernatan layer were separated and the solvent was evaporated under N_2 gas flux. The fatty acid methyl esters were then analyzed by gas chromatography.

GC analysis

A Shimadzu-GC-9AM equipped with CP-SIL-88 column (30 m × 0.30 mm id) and flame ionization detector (FID) was utilized. The injector and detector temperature were 230°C, while column temperature was previously held at 120°C, then programmed to 200°C at 8°C/min. N_2 was used as a gas carrier.

RESULTS AND DISCUSSION

Fatty acid composition of palm stearin, coconut oil and fatty acid methyl esters of coconut oil

Palmitic acid was the major fatty acid in palm stearin, followed by oleic acid (Table 1). This result was in accordance with the earlier report by Ibrahim et al. (2008) that palm stearin from Malaysia contain palmitic acid around 52%.

As shown in Table 1, lauric acid was the main fatty acids in both coconut oil and fatty acid methyl ester of coconut oil. These were similar to the result of Marina et al. (2009), who reported that the lauric acid of coconut oil was about 46.64 to 48.03%. Thus, we consider that palm stearin and coconut oil were good source of 2-monopalmitin and medium chain fatty acids, respectively.

Fatty acid profile of purified 2-monoglycerides

From the TLC, five spots were obtained, which presumably were spot of triglycerides at the top, followed by free fatty acids, two spots of diglycerides, and the remaining spot was monoglycerides at the lowest. From TLC scanner, it was indicated that the monoglyceride spot constituting 40.45% of the entire components as previously reported (Karouw et al., 2012). We attempted to separate the desired product, the 2-monoglycerides, by scrapping and analyzing the fatty acid composition.. According to the fatty acid profile data (Table 2), the hydrolyzed 2-monoglyceride spot palmitic and oleic acids constitued of 55.21 and 35.26%, respectively. We consider

Table 2. Fatty acid profile of monoglyceride hydolysis product of palm stearin, before and after purification.

Fatty acid	% Fatty acid	
	Before purification[a]	After purification (2-MG)[b]
C16:0	58.38 ± 0.92	55.21 ± 0.04
C18:0	3.70 ± 0.06	2.82 ± 0.06
C18:1	31.20 ± 0.33	35.26 ± 0.19
C18:2	6.72 ± 0.65	6.92 ± 0.31

[a]Obtained by hydolysis of palm stearin using porcine pancreatic lipase type II as biocatalyst, enzyme was 5.0% by weight of substrate, substrate: phospate buffer ratio = 10:4, pH 6.5. Incubation was at 37°C for 42 h in a waterbath shaker at 80 stroke/min. [b] 2-MG was purified by TLC on silica gel F254 plate, developed in petroleum eter:diethyl ether: acetic acid = 60:40:1.

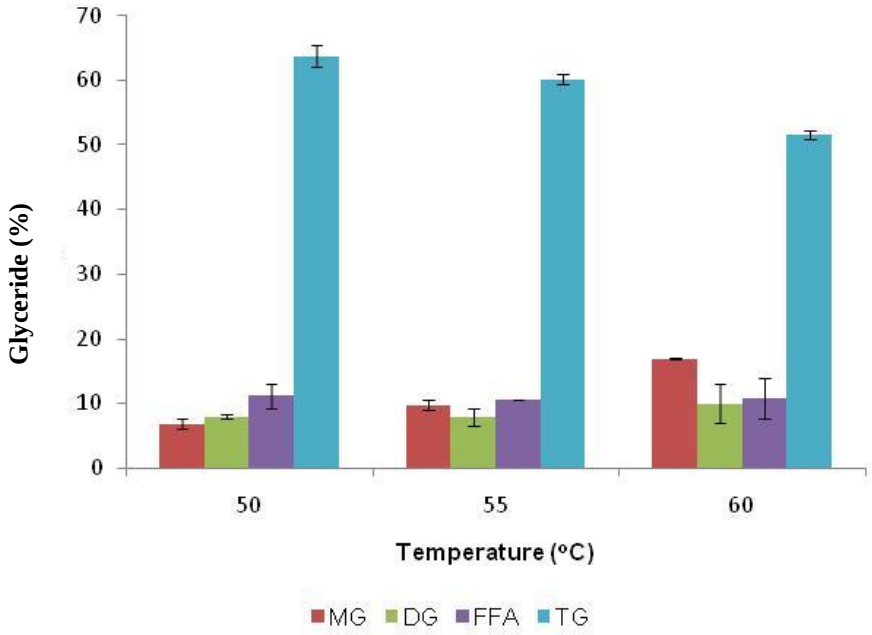

Figure 1. Effect of temperature on glycerides composition by interesterification of 2-monoglyceride and FAME with lipozyme RM IM as biocatalyst. A mixture of MG/FAME (1:3, w/w); with enzyme concentration was 10% by weight of total substrate. Incubate in a waterbath shaker at 120 stroke/min for 24 h. FAME, fatty acids methyl ester; MG, monoglycerides; DG, diglycerides; FFA, free fatty acids; TG, triglycerides.

that the 2-monoglyceride containing mainly palmitic and oleic acids is similar to human milk fat, therefore suitable for synthesis of human milk fat analog.

Effect of interesterification temperature on HMF analog content

Human Milk Fat analog (HMF analog) was the triglycerides obtained during interesterification of specific fatty acid methyl esters and 2-monoglycerides. The temperature ranges were varied at 50, 55 and 60°C refering to previous workers who reported the optimum condition of Lipozyme RM activity for synthesis of structured lipids, were between 50 to 60°C (Ilyasoglu et al., 2011; Kim et al., 2004; Maduko et al., 2008; Mu et al.,1998; Sahin et al., 2006).

An increase of interesterification reaction temperature from 50 to 60°C slightly decreased the triglycerides (HMF analog) from 63.75 to 60.18%, but significantly increased Monoglycerides (6.81 to 16.84%) and diglycerides (7.86 to 9.97%) (Figure 1). An elevation of reaction temperature

Table 3. Glycerides profile of esterified product during interesterification of 2-monoglyceride and FAME with lipozyme RM IM as biocatalyst on various reaction time.

Reaction time (h)	% Glyceride			
	MG	DG	FFA	TG
6	12.68 ± 1.56	13.64 ± 1.91	14.31 ± 0.66	57.18 ± 1.32
12	14.76 ± 4.31	8.31 ± 0.62	15.07 ± 2.24	60.24 ± 0.37
18	15.87 ± 1.95	6.23 ± 2.35	18.32 ± 3.32	54.87 ± 1.95
24	15.58 ± 0.52	7.57 ± 0.73	16.82 ± 0.56	52.41 ± 2.04

A mixture of MG/FAME (1:3, w/w); enzyme concentration was 10% by weight of total substrate. Incubation was at 50°C in a waterbath shaker at 120 stroke/min. FAME, fatty acids methyl ester; MG, monoglycerides; DG, diglycerides; FFA, free fatty acids; TG, triglycerides.

increased enzyme activity which lead to raising the triglyceride (the HMF analog) result. In contrast, if the reaction temperature was higher than the optimum enzyme temperature (50°C), it would markedly reduced the amount of HMF analog result. Reaction at higher temperature decreased enzyme stability caused by weakening of the intermolecular forces responsible of its three-dimensional structure, leading to a reduction in its catalytic capacity (Illanes et al., 2008).

These results were comparable to those of Mu et al. (1998), who interesterified sunflower oil and caprylic acid by immobilized lipase from *R. miehei,* that the synthesized triglyceride increased as the temperature elevated from 40 to 60°C. The results also in line with Subroto et al. (2008), who synthesized stuctured lipid by enzymatic interesterification of fish oil with lauric acid using immobilized lipase from *Mucor miehei*. They found that increasing of reaction temperature from 20 to 40°C tends to increase the triglyceride result, meanwhile at temperature above 40°C the triglyceride result decreased. Ilyasoglu et al. (2010) reported that an increase of the temperature above 57°C decreased the enzymatic interesterification reaction on capric and caprilic acids substrate to tripalmitin. These results were in accordance with the earlier report by Tan et al. (2011) who synthesized natural aroma-active 2-phenylethyl esters from coconut cream using five commercial lipases. They found that at temperature higher than 60°C leading to about 50% reduction in the yield. The results of this study, therefore, suggest that the condition to synthesis HMF analog from fatty acids methyl ester and 2-monoglyceride using lipozyme RM IM as biocatalyst was at 50°C. The optimum temperature of lipozyme RM IM for interesterification reaction was obviously depend on the substrate in use.

Effect of interesterification reaction time on HMF analog produced

The reaction mixture consisted of purified 2-MG and fatty acid ester methyl (FAME) containing 5.14% of triglyceride and high percentage of FAME (94.86%). During the interesterification reaction, the observed remaining FAME were 2.18, 3.25, 2.43 and 1.35% at 6, 12, 18 and 24 h of reactions, respectively.

The results showed that the HMF analog markedly increased during the first 6 h of reaction (57.18%) and continuously increased up 60.24% at 12 h (Table 3). It indicated that during the first 6 h, the interesterification reaction between monoglyceride and fatty acid methyl ester occured and reached its maximum at around 12 h. The amounts of HMF analog significantly decreased from 60.24 to 52.41%, after 12 and 24 h of reaction times, respectively.

In contrast, at the same time, the monoglyceride increased slightly to reach approximately 14.76 and 15.58%.

This results indicate that the interesterification reaction started at the early stage of reaction (6 h), however, after 12 h, the reaction was dominated by hydrolysis reaction of HMF analog to diglyceride and hydrolysis of diglyceride to monoglyceride. The diglyceride decreased until 18 h and then increased slightly to reach approximately 7.57% at 24 h. The diglyceride may be formed through hydrolysis of triglyceride or interesterification of monoglyceride. Hydrolysis of HMF analog triglyceride produced diglyceride and free fatty acid, meanwhile re-esterification of monoglyceride and free fatty acid formed diglyceride.

These results were in line with the finding of Willis and Marangoni (2002) and Xu et al. (1998) who mentioned that free fatty acids in the system can promote the re-esterification of monoglyceride and fatty acids to form diglyceride.

These results were similar to the report of Subroto et al. (2008) who reported that prolonging the interesterification reaction time for more than 6 h would turn the reaction from synthesis of structured lipid to hydrolysis of triglyceride as indicated by the elevation of monoglyceride. The presented results, therefore, suggest that the condition to produce HMF analog from fatty acids methyl ester and 2-monoglyceride by using lipozyme RM as biocatalyst was at 50°C for 12 h.

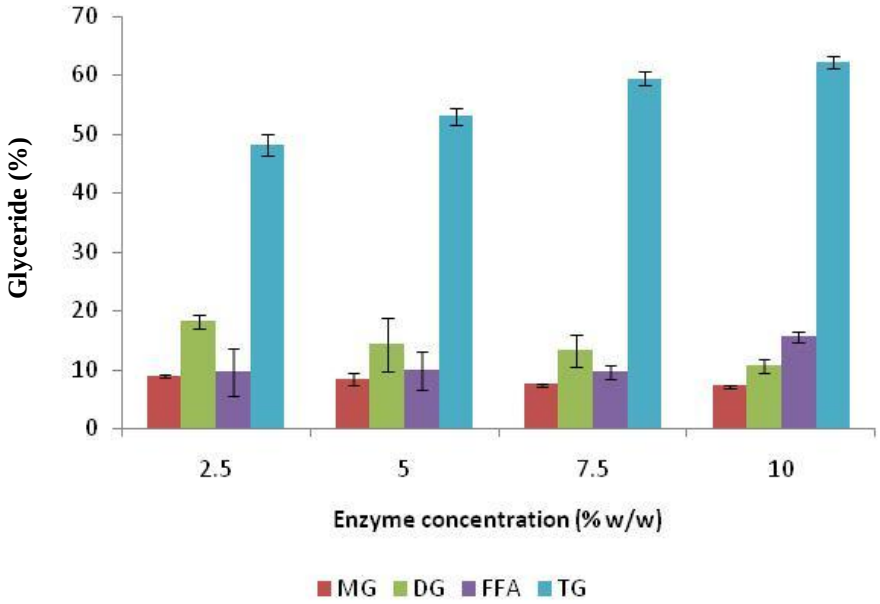

Figure 2. Effect of enzyme concentration on glycerides composition by interesterification of 2-monoglyceride and FAME with lipozyme RM IM as biocatalyst. A mixture of MG/FAME (1:3, w/w). Enzyme amount was based on weight of total substrate (2.5, 5.0, 7.5 and 10.0%, respectively). Incubation was at 50°C for 12 h in a waterbath shaker at 120 stroke/min. FAME, fatty acids methyl ester; MG, monoglycerides; DG, diglycerides; FFA, free fatty acids; TG, triglycerides.

Effect of enzyme concentration during interesterification on HMF analog content

The HMF analog content increased with the raise of enzyme concentration (Figure 2). The HMF analog content were 48.21, 53.06, 59.38 and 62.25% at enzyme concentration of 2.5, 5.0, 7.5 and 10.0% by weight of total substrate, respectively. As the consequence, the diglyceride and monoglyceride decreased to reach approximately 7.19 and 10.58%, respectively at the enzyme concentration 10.0% (w/w). The highest HMF analog content (62.25%) was achieved at 10.0% of enzyme concentration. At the same length of reaction time, rising of enzyme concentration resulted in increasing product concentration. As more enzymes were added, more active sites of the enzyme were accessible for the formation of the substrate-enzyme complex, leading to increasing of desired products (Rahman et al., 2008). However, a further increase of enzyme amount in the system caused reduction of the products due to as the high amount of enzyme was added, the viscosity of the reaction medium was increased, then led to the less effective transfer of the substrates to the active sites of the excess enzyme molecules inside the bulk of enzyme particles (Hari Krishna et al., 2001; Yadav and Trivedi, 2003).

These were similar to the results of Hamam et al. (2005) on synthesis of structured lipids from eicosapentaenoic acids and Laurical, a high laurate canola oil, using lipase from *Pseudomonas* sp. (PS-30) as biocatalyst. They found that increasing enzyme concentration from 2 to 4%, incorporation of eicosapentaenoic acids into laurical increased gradually, reaching maximum (66.9%) at 4% of enzyme. Then, the incorporation slightly decreased at enzyme concentration above 10%. On synthesis of structured lipid from high eicosapentaenoic and docosahexaenoic acids using RM IM as biocatalyst (Jennings and Akoh, 1999), it was found that as the enzyme concentration increased, the incorporation of capric acid also increased and the higest incorporation (41.4%) occured at 10% of enzyme concentration. Sun et al. (2012) have synthesized octanoic acid ester from coconut oil and fusel alcohol, using lipozyme TL IM. They reported that the formation of octanoic esters rised at enzyme concentration in the range of 5 to 15%, then a further increased of enzyme amount from 5 to 15% caused a reduction of the octanoic ecid esters. Therefore, we concluded that, the condition for interesterification of high palmitic 2-monoglycerides and high lauric fatty acid methyl esters to produce HMF analog by using lipozyme RM IM as biocatalyst was at 50°C for 12 h of reaction time and 10.0% (w/w) of enzyme concentration.

Fatty acid composition of HMF analog

The human milk fat analog, result from interesterification

Table 4. Fatty acids profile of human milk fat analog and human milk fat.

Fatty acid	% Fatty acid	
	HMF analog[a]	Human milk fat[b]
C10:0	4.49 ± 0.43	2.35
C12:0	39.37 ± 0.92	13.82
C14:0	16.06 ± 0.39	12.12
C16:0	24.33 ± 1.59	23.02
C18:0	5.37 ± 0.69	4.75
C18:1	8.98 ± 0.74	21.85
C18:2	1.40 ± 0.11	ND
Total MCFA	43.56	16.17

[a]Synthesized by interesterification of monoglyceride and FAME with lipozyme RM IM as biocatalyst (MG/FAME = 1:3 w/w; Incubation was at 50°C and 10% of enzyme for 12 h in waterbath shaker (120 stroke/min). ND: Not detected. [b]Human milk fat from breastfeeding mother in Philippine (Yuhas et al., 2006).

process of 2-monoglyceride derived from palm stearin and fatty acid methyl ester from coconut oil, was found to be rich of medium chain fatty acid. The fatty acid profiles of the HMF analog compare to fatty acid of human milk fat are presented in Table 4. The palmitic acid content, thought to be located in the sn-2 position of the HMF analog is comparable to that of human milk fat. However, oleic acid, which also thought to be in the sn-2 position, was noticeably lower than that in the human milk fat. The MCFA (lauric acid from coconut oil) was succesfully incorporated into the triglyceride. Ilyasoglu et al. (2010) reported that human milk fat analog synthesized from tripalmitin and Neobee (the mixture of medium chain fatty acids) contained MCFA of 23.4 g/100 g.

These results were similar to the earlier report by Li et al. (2009), who reported that human milk fat substitute synthesized from butterfat, rapeseed oil, and soybean oil using lipozyme RM IM, contained 24.5% of palmitic acid. The results also in line with Maduko et al. (2008), who interesterified coconut oil, soy bean oil, safflower oil, fish oil, and tripalmitin using lipozyme RM IM. They obtained human fat analog with palmitic acid around 23.0%. Kuipers et al. (2012) and Yuhas et al. (2006) reported that palmitic acid content in milk fat of breastfeeding mothers in sub-Sahara Africa and Philppine were 21.2 to 26.4% and 23.02%, respectively.

According to the results, therefore, we consider that the human milk fat analog synthesized from fatty acid methyl ester of coconut oil and palm stearin 2-monoglyceride catalyzed by lipozyme RM IM provides triglycerides rich of palmitic in the sn-2 and MCFA lauric in the sn-1 and sn-3 positions. The existence of palmitic in the sn-2 position was aimed to be similar to human milk fat, and the MCFA lauric acid in the sn-1 and sn-3 positions was expected to provide fatty acid energy that will be readily utilized by the body and not stored in the fat muscle.

Conclusion

The purified 2-monoglycerides from palm stearin consisted of palmitic and oleic acids 55.21 and 35.26%, respectively. The condition for interesterification of high palmitic 2-monoglycerides and high lauric fatty acid methyl esters to produce HMF analog by using lipozyme RM IM as biocatalyst was at temperature of 50°C for 12 h of reaction time and 10.0% (w/w) of enzyme concentration. The HMF analog produced under these condition contained MCFA of 43.86% and the main fatty acid constituent was lauric acid of 39.37%, which is expected to supply non fattening energy to baby. The palmitic acid content in the sn-2 position was around 24.18%, similar to human milk fat, which is expected to provide similar physiological performance.

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