

Research Article

Preparation of human milk fat substitutes from basa catfish oil: Combination of enzymatic acidolysis and modeled blending

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Human milk fat substitutes (HMFSs) were prepared from basa catfish oil by combination of Lipozyme RM IM-catalyzed acidolysis and physical blending. In acidolysis step, sesame oil fatty acids were used as acyl donors to replace palmitic acid (PA) at sn-1, 3 positions of basa catfish oil, and the enzymatic product had 23.8% PA, 48.3% PA at sn-2 position (sn-2 PA), and 67.7% distribution probability of sn-2 PA among total PA (%sn-2 PA) under the selected conditions: Enzyme load, 8 wt%; temperature, 40°C; substrate molar ratio, 1:3; water content, 3.5 wt%; and reaction time, 2 h. In blending step, with fatty acid profiles of human milk fat (HMF) as a preferable goal, selected oils were added to the enzymatic product, and the desirable formula predicted by the blending model which guaranteed the maximum addition constituted enzymatic product/flaxseed oil/sunflower oil/palm kernel oil/palm stearin/algal oil/microbial oil at a mole ratio of 1: 0.1319: 0.0353: 0.1775: 0.0674: 0.0078: 0.0128. The blending product had 21.5% PA, 39.0% sn-2 PA, and 60.4% %sn-2 PA, respectively. The similarity of the product to HMF was assessed by the evaluation model, and the achievement of high scores indicated it has potential for use as fat substitute in infant formulas.

Practical applications: Basa catfish oil was found to contain around 30% total PA with more than 45% sn-2 PA, which is a new alternative starting material suitable for HMFS production. Basa catfish is widely cultured in southern Vietnam in floating cages with annual production of 15 000 tonnes. Preparation of HMFSs from basa catfish oil is thus important for the development of HMFSs. This process reported preparation of HMFSs by combination of enzymatic acidolysis and modeled blending, with the attributes of lower cost and higher similarity, has great potential for industrial uses.

Keywords: Acidolysis / Basa catfish oil / Blending / Human milk fat substitutes / Lipozyme RM IM

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1 Introduction

Fat in human milk, termed as human milk fat (HMF), accounting for 3–5%, was the major energy source (>50%) for newborn infants [1, 2]. Differing from other fats and oils, HMF has special chemical composition and structure which

were derived from evolution. The most unique attribute was its palmitic acid (PA) distribution. HMF contains 20–30% palmitic acid, more than 60% of which was located at sn-2 position [3, 4]. Therefore, large amount of triacylglycerols (TAG) in HMF existed in the form of USU, such as 1,3-dioleoyl-2-palmitoylglycerol (OPO) and 1-oleoyl-2-palmitoyl-3-linoleoylglycerol (OPL), which were of great importance for the absorption and metabolism of fat in infants [5–8]. Besides 10–30% of the ingested TAG were hydrolyzed to sn-1, 2 diacylglycerols (DAG) by gastric lipase (a sn-3 preferential lipase), most of TAGs are digested by pancreatic lipase (a sn-1,3-specific lipase) in the small

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Abbreviations: HMF, human milk fat; HMFSs, human milk fat substitutes; PA, palmitic acid; TAG, triacylglycerols; TLC, thin layer chromatography

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intestine to sn-2 monoacylglycerols (MAG) [9]. The sn-2 MAG can be absorbed in the original form and esterified with free fatty acids into TAG in epithelial cells, and 60–70% of sn-2 fatty acids can remain in the original position after the esterification. Therefore, this TAG structure with PA at sn-2 position would avoid the formation of soaps and loss of energy [10]. Additionally, the mean diameter of the chylomicrons in the OPO group was larger than that in the OOP group, suggesting that OPO was absorbed and transported more effectively than OOP, indicating the intramolecular structure of HMF might have great influence on its metabolism in infants [11].

To our best knowledge, the raw materials currently used for human milk fat substitute (HMFS) preparation were lard, palm stearin, and butterfat. Lard is the natural fat with the most similar chemical composition and molecular structure to HMF [12–14]. It had more than 65% of palmitic acid at sn-2 position, and thus high content of OPO. However, due to religious reasons, some people cannot eat food derived from pigs, and thus lard as raw material for HMFS preparation is limited. As far as we know, only meiji infant formula from Japan uses lard in its product. The production of HMFSs is industrialized by use of palm stearin as the starting material by Lourdes Crow Kelan (Betapol[®]) and Advanced Lipids (Infat[®]) [15]. However, palm stearin has high content of PA and the process for preparation of HMFSs required high substrate ratios, which resulted in the difficulty for subsequent deacidification and thus high production cost [16–18]. As for butterfat, due to low content of PA at sn-2 position, it is hard to modify it with high similarity to HMF [19]. Therefore, it is necessary to find another fat, which has high content of sn-2 PA yet low content of PA and can be widely accepted. After extensive analysis of different fats and oils, basa catfish oil was found to have high content of sn-2 PA (>45%) and low content of PA (around 30%), which is similar to HMF. It could be the best starting material for HMFS preparation, not only for its palmitic acid distribution, but also for the existence of a certain amount of PUFA.

Therefore, the objective of this study was to use basa catfish oil as the starting material to prepare HMFSs. The preparation was carried out by a two-step process, including enzymatic acidolysis of the fish oil to decrease the content of PA and physical blending to adjust the composition and distribution of other fatty acids. In the acidolysis reaction, the parameters including enzyme load, substrate ratio, temperature, and water content were considered.

2 Materials and methods

2.1 Materials

Lipozyme RM IM and Novozyme 435 were purchased from Novozymes A/S (Bagsvaerd, Denmark). Pancreatin (porcine

pancreas) powder was bought from Sigma Corp. (USA). Basa Catfish oil (BCFO) was given by Zhonghai Ocean Science & Technology Co., Ltd., and sesame oil (SO), flaxseed oil (FSO), sunflower oil (SFO), palm kernel oil (PKO), and palm stearin (PS) were provided by Shanghai Kerry Oils & Grains Industries Co., Ltd. (Shanghai, China). Microbial oil (MO) rich in AA and algal oil (AO) rich in DHA was provided by Fuxing Biotechnology Co., Ltd. (Wuhai, Hubei). Fatty acid profiles of BCFO, SO, FSO, SFO, PKO, PS, MO, and AO are shown in Table 1. TAG standards including 1,3-dioleoyl-2-palmitoylglycerol (OPO), 1,2-dioleoyl-3-palmitoylglycerol (OOP), 1,2-dipalmitoyl-3-oleoylglycerol (PPO), 1,3-dipalmitoyl-2-oleoylglycerol (POP), triolein (OOO), 1,2-dioleoyl-3-stearoylglycerol (OOS), 1,3-stearoyl-2-oleoylglycerol (SOS), 1,3-stearoyl-2-oleoylglycerol (SSO), tripalmitin (PPP), and 1-palmitoyl-2-oleoyl-3-stearoylglycerol (POS) were purchased from Larodan Fine Chemicals AB (Malmö, Sweden). Silicic acid 60G TLC plates (10 × 20 cm) were purchased from Shanghai Shangbang Co., Ltd. (Shanghai, China). Acetonitrile and isopropanol were of high-performance liquid chromatography (HPLC) purity. Hexane, hydrochloric acid, glacial acetic acid, methanol, sulfuric acid, and ethyl ether were of analytical grade.

2.2 Preparation of free fatty acids

Free fatty acids were prepared from sesame oil as described by Senenayeke and Shahidi [20]. Twenty five grams of oil was saponified by mixing with KOH (5.75 g), water (11 mL), and 95% v/v aqueous ethanol (66 mL) for 1 h at 60°C by refluxing. Distilled water (50 mL) and hexane (2 × 100 mL) were then added to the mixture. The organic layer with unsaponifiable matters was discarded, and the aqueous layer containing the saponifiable matters was acidified with 3 M HCl to a pH of 1.0. The fatty acids were released after acidification and then extracted with hexane (50 mL). The hexane layer was dried over anhydrous sodium sulfate, and the fatty acids were then recovered by removal of the solvent with a rotary evaporator at 40°C.

2.3 Enzymatic acidolysis

Basa catfish oil (5 g) was mixed with free fatty acids from sesame oil at different substrate molar ratios ranging from 4 to 12 in 25 mL round-bottom flasks, and then the lipase (5–14 wt%, by the weight of total substrates) with water content from 3.5 to 17 wt% (by the weight of enzyme) was added to start the reaction. Samples were flushed with nitrogen and incubated in a water bath with magnetic agitation at 250 rpm.

2.4 Triglyceride isolation

A 50 µL aliquot of the reaction product was taken from the reaction system and isolated by thin layer chromatography

Table 1. Fatty acid profiles of basa catfish oil (BCFO), sesame oil (SO), flaxseed oil (FSO), sunflower oil (SFO), palm kernel oil (PKO), palm stearin (PS), algal Oil (AO), and microbial Oil (MO)

Fatty acid	BCFO			SO	FSO	SFO	PKO	PS	AO	MO
	Total	sn-2	%sn-2 ^a	Total	Total	Total	Total	Total	Total	Total
C8:0								4.4		
C10:0								4.8		
C12:0	0.3	0.4	47			0.15	1.3	55.7		
C14:0	4.6	4.2	30.5			1.17	1.7	15.1	8.7	0.4
C16:0	32.8	49.3	50.2	9.2	5.61	61.46	6.8	6.4	24.7	10.9
C18:0	8.7	5.4	20.6	5.5	3.59	5.2	5.2	1.1	0.7	6.1
C18:1n-9	38.9	26.3	22.5	39.4	20.02	25.17	25.4	10.2	1.4	8.6
C18:2n-6	9.7	9.2	31.4	44.2	16.64	5.91	59	2.1	0.4	4
C18:3n-3	0.4	0.5	38.1	0.4	42.66	0.09	0.6		0.3	2.8
C20:0	0.6	0.4	24		0.46				0.4	3.6
C20:3n-6	0.3	0.3	26.2		0.17				0.9	4.5
C20:4n-6	0.2	0.1	20.5						0.7	49
C22:0	0.5	0.2	12.6						0	4.4
C22:5n-3	0.1	0.1	41.9						15.4	
C22:6n-3	0.1	0.1	37.4						42	

^a The percentage of fatty acids located at the sn-2 position was calculated as $\text{sn-2} \times 100\% / (3 \times \text{total})$.

(TLC) plates with hexane/diethyl ether/acetic acid (80:20:1, v/v/v) as the developing solvent [21]. The plates were then sprayed with 0.2% 2, 7-dichlorofluorescein in methanol and visualized under UV light. The band corresponding to TAG was scraped off and methylated with 3 mL of 4% H₂SO₄ in methanol at 90°C for 20 min under nitrogen. The FA methyl esters were extracted twice with 2 mL of hexane, dried with anhydrous sodium sulfate, and concentrated with nitrogen.

2.5 Fatty acid composition analysis

The fatty acid composition of the products was analyzed with a GC-14B gas chromatograph, equipped with a flame ionization detector (Shimadzu, Tokyo, Japan) and a fused-silica capillary column (PEG-20M, 30 m × 0.32 mm × 0.5 μm). The column was initially held at 100°C for 4 min, followed by temperature programming to 180°C at the rate of 15°C/min, then held at 180°C for 4 min, and raised to 215°C at the rate of 4°C/min. The injection port and detector temperatures were both set at 250°C. The fatty acid methyl esters were identified by comparison of retention time with the standards, and the relative contents expressed as mole percent were then calculated.

2.6 Pancreatic lipase hydrolysis

TAGs were isolated by TLC as described above, and the band corresponding to TAGs was scraped off and extracted twice with 2 mL of ethyl ether. TAGs were obtained after removal of solvent by nitrogen concentration. Hydrolysis of TAGs was carried out according to the method described by

Luddy et al. [22]. One milliliter of 1 M Tris-HCl buffer (pH 8.0), 0.25 mL of 0.05% bile salts, 0.1 mL of 2.2% CaCl₂, and 10 mg of pancreatic lipase were added to the TAGs. The mixture was incubated in a water bath at 40°C for 3 min with vigorous shaking, and then 1 mL of 6 M HCl and 2 mL of diethyl ether were added and centrifuged. Diethyl ether was dried by anhydrous sodium sulfate and evaporated by nitrogen to 200 μL. The hydrolytic products were separated on TLC plates, and the developing solvent system was hexane/diethyl ether/acetic acid (50:50:1, v/v/v). The band corresponding to sn-2 MAG identified by the 2-oleoylglycerol was scraped off, methylated, and analyzed as mentioned above.

2.7 Deacidification by molecular distillation

Molecular distillation (KDL1, UIC GmbH, Alzenau, Germany) was used to remove the fatty acids in the products with the following conditions: Evaporator temperature, 185°C; condenser temperature, 40°C; heat exchanger temperature, 60°C; rotation speed of the wiped film, 120 rpm; feeding speed, 2 mL/min; absolute pressure, 2 Pa; preheating temperature, 50°C; condensing temperature, 50°C. The heating of the evaporator was provided by the jacket circulated with heated oil from an oil bath.

2.8 Physical blending

According to the fatty acid composition and distribution of HMF [3], the enzymatic product was blended with FSO, SFO, PKO, PS, MO, and AO at different ratios. The fatty

acid profiles of the blended oils were calculated by our previously established physical blending model [21].

$$\text{FA}\% = \frac{Y_1 + \sum_{i=2}^n Y_i X_i}{1 + \sum_{i=2}^n X_i} \quad (1)$$

$$\text{sn-2FA}\% = \frac{Y_{1(\text{sn-2})} + \sum_{i=2}^n Y_{i(\text{sn-2})} X_i}{1 + \sum_{i=2}^n X_i} \quad (2)$$

$$\text{sn-1, 3FA}\% = \frac{3 \times \left(Y_1 + \sum_{i=2}^n Y_i X_i \right) - \left(Y_{1(\text{sn-2})} + \sum_{i=2}^n Y_{i(\text{sn-2})} X_i \right)}{2 \times \left(1 + \sum_{i=2}^n X_i \right)} \quad (3)$$

$$\% \text{sn-2FA} = \frac{Y_{1(\text{sn-2})} + \sum_{i=2}^n Y_{i(\text{sn-2})} X_i}{3 \times \left(Y_1 + \sum_{i=2}^n Y_i X_i \right)} \times 100 \quad (4)$$

Where Y_1 and Y_i are the percentages of a fatty acid species among three acylglycerol positions in the enzymatic product and selected oil; $Y_{1(\text{sn-2})}$ and $Y_{i(\text{sn-2})}$ are the percentages of a fatty acid species among sn-2 fatty acids in the enzymatic product and selected oil; X_i is the molar ratio between the selected oil and the enzymatic product, in which the enzymatic product is set to be 1; n is the oil species.

The final amount of the product can be described as follows:

$$M = X_1 \left(M_1 + \sum_{i=2}^n X_i M_i \right) \quad (5)$$

Where M is the final amount of the product; X_1 is the moles of the enzymatic product and M_1 is its molecular weight; M_i is the molecular weight of the selected oil.

To guarantee the quality of the final product and the maximum yield, Matlab R2010a (MathWorks, Natick, MA) was used to optimize the entire blending process.

2.9 Similarity evaluation

The degrees of similarity of HMFSs were evaluated by our established model from the perspective of TAG profiles by

employment of four indicators, including total (C10:0, C12:0, C14:0, C16:0, C16:1, C18:0, C18:1, and C18:2) and sn-2 fatty acid (C12:0, C14:0, C16:0, C16:1, C18:0, C18:1, and C18:2) composition, and PUFA (C18:3, C20:2, C20:3, C20:4, C20:5, C22:2, C22:4, C22:5, C22:6) and TAG (OLaLa, MMLa, LLaO, MOLA, PMLa, LaOO, POLa, MPL, SMM, POL, PPL, PPM, OOO, POO, PPO, and POS) composition (obtained from RP-HPLC) [3, 23]. The results from four aspects of HMFSs could precisely reflect their degrees of similarity in chemical composition to HMF. The model was given as follows:

$$G_{\text{FA/sn-2FA/PUFA/TAG}} = 100 - \sum_{i=1}^n E_{i(\text{FA/sn-2FA/PUFA/TAG})} \quad (6)$$

$$E_{i(\text{FA/sn-2FA/PUFA/TAG})} = 100 \times \left(C_{i(\text{FA/sn-2FA/PUFA/TAG})} \frac{D_{i(\text{FA/sn-2FA/PUFA/TAG})}}{\sum_{i=1}^n D_{i(\text{FA/sn-2FA/PUFA/TAG})}} \right) \quad (7)$$

$$C_{i(\text{FA/sn-2FA/PUFA/TAG})} = \frac{|B_{i(\text{FA/sn-2FA/PUFA/TAG})} - A_{i(\text{FA/sn-2FA/PUFA/TAG})}|}{A_{i(\text{FA/sn-2FA/PUFA/TAG})}} \quad (8)$$

Where $G_{\text{FA/sn-2FA/PUFA/TAG}}$ is the degree of similarity of HMFSs to HMF in the aspect of total fatty acid composition, the percentages of fatty acids in the sn-2 position relative to the three acylglycerol positions, PUFA or TAG composition for the n different fatty acyl species; $E_{i(\text{FA/sn-2FA/PUFA/TAG})}$ is the deducted degree of similarity of the content of fatty acyl species i , the percentage of fatty acyl species i in the sn-2 position relative to its three acylglycerol positions, content of polyunsaturated fatty acyl species i or triacylglycerol i of HMFSs that is outside the range of that of HMF; $D_{i(\text{FA/sn-2FA/PUFA/TAG})} / \sum_{i=1}^n D_{i(\text{FA/sn-2FA/PUFA/TAG})}$ is the weight of the fatty acyl species i , sn-2 fatty acyl species i , polyunsaturated fatty acyl species i , or triacylglycerol i of HMF relative to its total amount; $C_{i(\text{FA/sn-2FA/PUFA/TAG})}$ is the floating coefficient, which is dependent on the content of fatty acyl species i , the percentage of fatty acyl species i in the sn-2 position relative to its three acylglycerol positions, content of polyunsaturated fatty acyl species i , or triacylglycerol i of HMFSs. $B_{i(\text{FA/sn-2FA/PUFA/TAG})}$ is the content of fatty acyl species i , the percentage of fatty acyl species i in the sn-2 position relative to its three acylglycerol positions,

content of polyunsaturated fatty acyl species i , or triacylglycerol i of HMFSs; $A_{i(\text{FA/sn-2FA/PUFA/TAG})}$ is the upper or lower limit of corresponding indicator i in HMF. When B is higher than the upper limit of the corresponding indicator in HMF, A is selected as the upper limit, and vice versa. If B is within the range, C is set to zero.

3 Results and discussion

Basa catfish oil has high content of sn-2 PA, but compared with HMF, it contains higher content of PA which leads to lower %sn-2PA, and lacks MCFA and some PUFA. Therefore, to increase its similarity to HMF, two-step method including enzymatic acidolysis to increase %sn-2 PA and physical blending optimized by mathematical model to adjust fatty acid profiles were used. In the enzymatic acidolysis step, fatty acids from sesame oil containing similar contents of oleic and linoleic acid were used as acyl donors.

3.1 Enzymatic acidolysis reactions

3.1.1 Effect of enzyme load

As the catalyst in acidolysis reactions, the amount of lipase has great influence on the reaction rate. Higher amount of lipase leads to shorter reaction time to reach equilibrium [24]. Additionally, acidolysis reaction was a two-step reaction, including hydrolysis and re-esterification [25]. Enzyme load has some influence on the amount of DAG, and thus on the rate of acyl migration. Therefore, considering the reaction efficiency and product quality, selection of enzyme load is necessary. The effect of enzyme load on the content of PA, %sn-2 PA as a function of time is shown in Fig. 1. As for the content of PA, the decrease rate was increased with the increase of enzyme load during the initial reaction stage. In terms of reactions with enzyme load of 2 wt%, the reaction

rate was slower than those of other enzyme loads, and the equilibrium was not reached until 6 h reaction. However, other reactions all reached equilibrium within 6 h, and reactions with enzyme load of 8, 11, and 14 wt% had the same equilibrium time of 2 h.

Before acidolysis reaction, basa catfish oil has low %sn-2 PA due to relatively high content of PA. The palmitic acid at sn-1, 3 positions was replaced by oleic and linoleic acids when the reactions were switched on and thus %sn-2 PA was increased. However, as the reactions proceeded, the content of sn-2 PA was decreased due to the occurrence of acyl migration and the content of PA reached equilibrium, which lead to decrease of %sn-2 PA after achievement of the peak value. Reactions with enzyme load of 2 wt% has low increase rate of % sn-2 PA and reactions with enzyme load of 8, 11, and 14 wt% has similar peak values. Therefore, after overall consideration, the enzyme load was selected as 8 wt% for further study.

3.1.2 Effect of temperature

Temperature imposes great influence on the rate of enzymatic acidolysis reaction. However, higher reaction temperature can result in higher acyl migration rate and enzyme deactivation rate [26, 27]. Therefore, the reaction temperature should be reduced as low as possible on the condition that reaction efficiency and product quality are guaranteed. The effect of temperature on the content of PA and %sn-2 PA as a function of time is shown in Fig. 2. The decrease rate of the content of PA increased with the increase of reaction temperature. As for reactions under temperatures of 40, 50, and 60°C, the equilibrium could be reached after 2 h reaction and as for reactions at 70°C, the time to reach equilibrium was 1 h. All reactions had similar contents of PA when the equilibriums were reached.

The value of %sn-2 PA was dependent on the contents of total PA and sn-2 PA, which were both affected by reaction

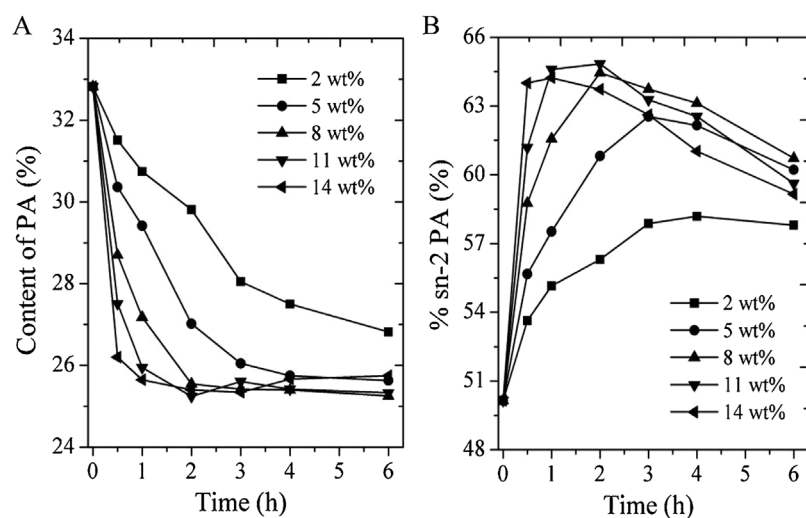


Figure 1. Effect of enzyme load on the contents of PA (A) and %sn-2 PA (B) as a function of reaction time. Reaction conditions: Temperature, 50°C; substrate molar ratio, 1:2; water content, 3.5 wt%.

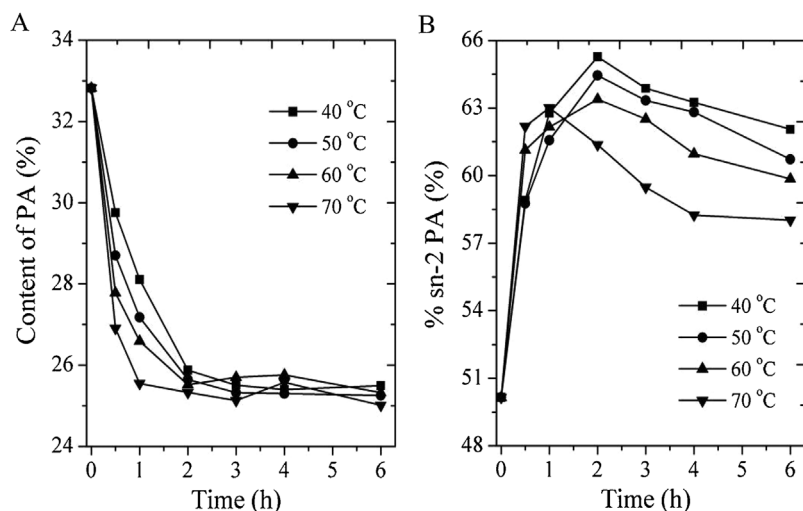


Figure 2. Effects of temperature on the contents of PA (A) and %sn-2 PA (B) as a function of reaction time. Reaction conditions: Enzyme load, 8 wt%; substrate molar ratio, 1:2; water content, 3.5 wt%.

temperature. Sn-1, 3 PA was replaced by other fatty acids in the acidolysis reactions, which led to the increase of %sn-2 PA. Similar to the effects of enzyme load, higher temperature lead to higher replacing rate of sn-1, 3 PA, namely, higher decrease rate of the content of PA. Therefore, the increase rate of %sn-2 PA rose with the increase of reaction temperature. However, acyl migration occurred during acidolysis reactions, which could explain the decreasing tendency of %sn-2 PA after the reaction equilibriums. The peak values of %sn-2 PA of reactions at temperatures of 40, 50, 60, and 70 °C were 65.27, 64.45, 63.39, and 63.01%, respectively, decreasing with the increase of temperature. Therefore, on the basis of the above-mentioned results, 40 °C was selected as the reaction temperature for the subsequent reactions.

3.1.3 Effect of substrate molar ratio

Lipase-catalyzed acidolysis is a reversible reaction. Enzyme load and temperature have influence on the reaction rate, that

is, the time to reach equilibrium; however, the conversion rate, that is, the amount of the replaced sn-1, 3 PA, is mainly decided by substrate molar ratio [28]. The effects of substrate molar ratio on the content of PA and %sn-2 PA as a function of time are shown in Fig. 3.

The decrease rate of the content of PA increased with the increase of substrate molar ratio, and reactions with higher substrate molar ratio has lower content of PA, which indicated more sn-1, 3 PA was replaced. However, the difference in the contents of PA of reactions with substrate molar ratios of 1:3 and 1:4 were not significant after the equilibrium was achieved, with the values of 24.09 and 24.27%, respectively. As for reactions with substrate molar ratios of 1:1–1:3, the reactions could reach equilibrium after 2 h reaction; however, for reactions with substrate ratios of 1:4, 3 h was required. More sn-1, 3 PA could be replaced under the conditions of higher substrate ratios, and thus %sn-2 PA increased with the increase of substrate molar ratio. When the reactions achieved equilibrium, the content

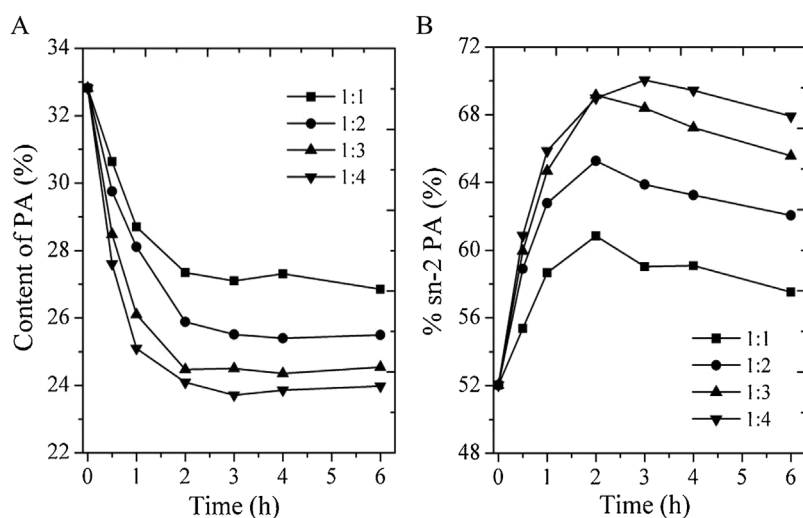


Figure 3. Effects of substrate molar ratio on the contents of PA (A) and %sn-2 PA (B) as a function of reaction time. Reaction conditions: Enzyme load, 8 wt%; temperature, 40 °C; water content, 3.5 wt%.

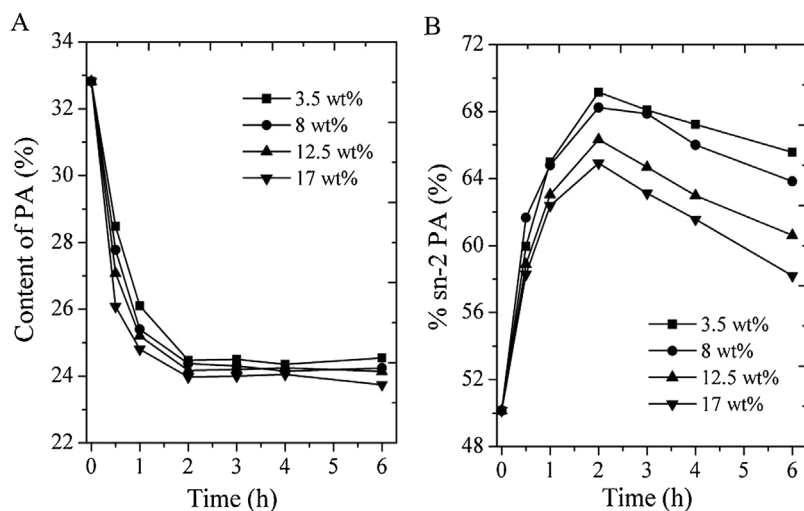


Figure 4. Effects of water content on the contents of PA (A) and %sn-2 PA (B) as a function of reaction time. Reaction conditions: Substrate molar ratio, 1:3; enzyme load, 8 wt%; temperature, 40°C.

of sn-2 PA decreased due to acyl migration and the content of PA kept constant, and thus %sn-2 PA decreased after the achievement of the equilibrium. The %sn-2 PA of reactions with substrate molar ratios of 1:1–1:3 reached their summits after 2 h reaction with values of 60.84, 65.27, and 68.39%, respectively, and the peak value of reactions with substrate molar ratio of 1:4 was 70.04% after 3 h reaction. Therefore, after comprehensive consideration, substrate molar ratio of 1:3 was chosen for further study.

3.1.4 Effect of water content

In the lipase-catalyzed reactions, water in the system is used to keep enzyme active and to be the reactant for hydrolysis

[29]. The DAG produced from hydrolysis is the intermediate for new TAG synthesis [30]. Therefore, the amount of DAG in the system has great influence on the reaction rate, which was mainly affected by water content. However, higher amount of DAG could lead to higher rate of acyl migration and more loss of neutral oil, which are both disadvantageous for the reaction. Therefore, selection of water content is of great importance for acidolysis reactions. The effect of water content on the content of PA and %sn-2 PA as a function of time is shown in Fig. 4. As water content increased, the decrease rates of content of PA were increased. However, the difference in the decrease rate was not significant. The reactions at different water content level could all achieve equilibrium after 2 h reaction with similar values of content of

Table 2. Fatty acid composition and distribution of the enzymatic and blending products under the optimized blending conditions

Fatty acid	Enzymatic product				Blending product			
	Total	sn-2	%sn-2 ^a	sn-1,3 ^b	Total	sn-2	%sn-2 ^a	sn-1,3 ^b
C8:0					0.6	0.0	2.3	0.8
C10:0					0.6	0.1	6.9	0.8
C12:0	0.6	0.4	22.1	0.7	7.5	5.6	25.0	8.4
C14:0	3.5	3.0	28.6	3.8	4.6	4.2	31.1	4.7
C16:0	23.8	48.3	67.7	11.5	21.5	39.0	60.4	12.8
C18:0	6.6	5.3	26.5	7.3	5.6	4.4	26.3	6.2
C18:1n-9	39.5	33.3	28.1	42.6	33.0	31.7	31.9	33.7
C18:2n-6	23.2	9.0	13.0	30.3	20.0	11.8	19.7	24.1
C18:3n-3	0.5	0.3	15.7	0.7	4.4	3.4	25.4	4.9
C20:0	0.2	0.3			0.3	0.3	33.2	0.3
C20:3n-6	0.1	0.1			0.2	0.2	33.6	0.2
C20:4n-6					0.5	0.4	27.2	0.6
C22:0					0.0	0.0	9.1	0.1
C22:5n-3					0.1	0.1	29.7	0.1
C22:6n-3					0.2	0.3	37.9	0.2

^a The percentage of fatty acids located at the sn-2 position was calculated as $\text{sn-2} \times 100\% / (3 \times \text{total})$.

^b FA composition at sn-1,3 positions was calculated as $(3 \times \text{total} - \text{sn-2})/2$.

PA. As shown in Fig. 4b, the higher water content in the system could result in lower peak values when reaction equilibrium was reached. Therefore, after overall consideration, water content was selected as 3.5 wt%.

Therefore, based on the above-mentioned results, the selected conditions for enzymatic acidolysis were as follows: Enzyme load, 8 wt%; temperature, 40°C; substrate ratio, 1:3; and water content, 3.5 wt%; reaction time, 2 h. Under these conditions, the fatty acid profiles and TAG composition of the enzymatic product are presented in Tables 2 and 3, respectively. Compared with fatty acid profiles of basa catfish oil, the content of sn-2 PA of the enzymatic product has been decreased from 49.3 to 48.3% due to the occurrence of acyl migration; however, the %sn-2 PA has been increased from 50.2 to 67.7%, due to the substitution of at sn-1, 3 PA in the acidolysis reactions. Compared with fatty acid profiles of HMF, the enzymatic product has similar content of PA, myristic acid, and stearic acid. However, higher oleic acid and lower medium chain and long-chain polyunsaturated fatty acid contents were also observed. Therefore, in order to increase the similarity of the enzymatic product to HMF, other methods have to be taken to further improve its fatty acid profiles.

3.2 Physical blending

Based on the difference of the fatty acid profiles between HMF and the enzymatic product, FSO, SFO, PKO, PS, AO, and MO rich in linolenic acid, linoleic acid, lauric acid, DHA, and AA, respectively, were chosen to add to the enzymatic product and the ratio was optimized by the previously established model [21]. Under this blending ratio, maximum yield could be obtained and high degree of similarity could also be ensured.

The optimized ratio obtained from the model were enzymatic product: FSO: SFO: PKO: PS: AO: MO = 1: 0.1319: 0.0353: 0.1775: 0.0674: 0.0078: 0.0128. The selected oils were blended according to the ratio and were then randomized by Novezyme 435. The randomized oils were added to the enzymatic product and the fatty acid profiles and TAG composition of the blending product are shown in Tables 2 and 3, respectively. Compared with the fatty acid profile of enzymatic product, the contents of C12:0, C14:0, and C18:3 were significantly increased due to the addition of FSO and PKO, and the contents of AA and DHA were also increased due to the addition of MO and AO.

3.3 Similarity evaluation

The degree of similarity of the blending product to HMF was evaluated by the established model from the aspects of total and sn-2 fatty acids, PUFA and TAG composition [23], and basa catfish oil and the enzymatic product were also compared. The evaluated results are presented in Table 4.

Table 3. TAG composition of basa catfish oil, enzymatic product, and blending oil under the selected conditions

TAG	BCFO	Enzymatic product	Blending oil
LaLaLa			1.7
LaLaM			1.0
LnLnLn			2.8
LLnLn			1.4
LLLn			0.2
LnLnO			1.5
LnLnP			0.6
LaLaO			0.2
LaMM			0.4
LLL		1.1	1.6
LnLO			0.6
LnLP			0.2
LaMO			0.1
LaPM			0.1
LnLS			0.3
LLO		2.7	2.9
LnOO			0.6
PLL		2.5	2.2
LaPO			0.1
PML	2.0		
PMM	0.6		
LnOP			0.3
LOO	1.3	9.3	7.2
LLS			0.1
LOP	4.7	8.5	8.4
PPL	15.5	9.3	7.6
SOL	7.4	2.4	1.8
OOO	5.4	11.5	8.8
POO	30.5	38.7	34.1
POP	20.5	5.0	5.3
PPP	2.1	0.0	1.1
SOO	2.3	6.7	5.0
POS	5.6	1.0	0.7
PPS	1.1	0.4	0.2

La, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid.

Compared with BCFO, after acidolysis reaction, the similarity in sn-2 fatty acid composition of the enzymatic product was reduced due to acyl migration, whereas the similarity in TAG composition was increased due to the

Table 4. Similarity evaluation of basa catfish oil, enzymatic product, and blending oil

Scores	BCFO	Enzymatic product	Blending product
G_{FA}	86.5	87.1	98.2
$G_{sn-2 FA}$	83.3	75.6	80.8
G_{PUFA}	55.5	30.8	60.9
G_{TAG}	31.2	47.8	53.3

substitution of palmitic acid at sn-1,3 position with oleic and linoleic acid. After blending, the degrees of similarity in FA, sn-2 FA, PUFA, and TAG composition were all increased, which indicates that the product was more suitable for use as fat substitute in infant formula.

4 Conclusions

In conclusion, the preparation of HMFSs from BCFO with high degree of similarity to HMF was achieved by enzymatic acidolysis and physical blending. In the acidolysis step, free fatty acids from RSO and SFO in the ratio of 1:1 was used as acyl donors to increase the %sn-2 PA, and the conditions were determined as enzyme load, 8 wt%; temperature, 40°C; substrate molar ratio, 1:3; and water content, 3.5 wt%; reaction time, 2 h. Under these conditions, the %sn-2 PA of the enzymatic product has been increased from 50.2 to 67.7%, and the content of linoleic acid has been increased from 9.7 to 23.2%. In the physical blending stage, based on the chemical composition of HMF, FSO, SFO, PKO, PS, AO, and MO were selected and their ratio to the enzymatic product were optimized by the physical blending model as enzymatic product: FSO: SFO: PKO: PS: AO: MO = 1: 0.1319: 0.0353: 0.1775: 0.0674: 0.0078: 0.0128. The degree of similarity of the final product to HMF in the aspects of FA, sn-2 FA, PUFA, and TAG composition was, 98.2, 80.8, 60.9 and 53.3, respectively.

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References

- [1] Jensen R. G., Lipids in human milk. *Lipids* 1999, 34, 1243–1271.
- [2] Jensen R. G., Ferris A. M., Lammi-Keefe C. J., Henderson R. A., Lipids of bovine and human milks: A comparison. *J. Dairy Sci.* 1990, 73, 223–240.
- [3] Zou X. Q., Guo Z., Huang J. H., Jin Q. Z., et al., Human milk fat globules from different stages of lactation: A lipid composition analysis and microstructure characterization. *J. Agr. Food Chem.* 2012, 60, 7158–7167.
- [4] Wang Y. H., Mai Q. Y., Qin X. L., Yang B., et al., Establishment of an evaluation model for human milk fat substitutes. *J. Agr. Food Chem.* 2009, 58, 642–649.
- [5] Filer L., Mattson F., Fomon S., Triglyceride configuration and fat absorption by the human infant. *J. Nutr.* 1969, 99, 293–298.
- [6] Mu H., Porsgaard T., The metabolism of structured triacylglycerols. *Prog. Lipid Res.* 2005, 44, 430–448.
- [7] Carnielli V. P., Luijendijk I., van Goudoever J. B., Sulkers E. J., et al., Feeding premature newborn infants palmitic acid in amounts and stereoisomeric position similar to that of human milk: Effects on fat and mineral balance. *Am. J. Clin. Nutr.* 1995, 61, 1037–1042.
- [8] Kennedy K., Fewtrell M. S., Morley R., Abbott R., et al., Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effects on stool biochemistry, stool characteristics, and bone mineralization. *Am. J. Clin. Nutr.* 1999, 70, 920–927.
- [9] Mu H., Hoy C. E., The digestion of dietary triacylglycerols. *Prog. Lipid Res.* 2004, 43, 105–133.
- [10] Osborn H., Akoh C., Structured lipids—novel fats with medical, nutraceutical, and food applications. *Compr. Rev. Food Sci. F.* 2002, 1, 110–120.
- [11] Aoe S., Yamamura J. I., Matsuyama H., Hase M., et al., The positional distribution of dioleoyl-palmitoyl glycerol influences lymph chylomicron transport, composition and size in rats. *J. Nutr.* 1997, 127, 1269–1273.
- [12] Yang T., Xu X., He C., Li L., Lipase-catalyzed modification of lard to produce human milk fat substitutes. *Food Chem.* 2003, 80, 473–481.
- [13] Nielsen N. S., Yang T., Xu X., Jacobsen C., Production and oxidative stability of a human milk fat substitute produced from lard by enzyme technology in a pilot packed-bed reactor. *Food Chem.* 2006, 94, 53–60.
- [14] Wang Y. H., Qin X. L., Zhu Q. S., Zhou R., et al., Lipase-catalyzed acidolysis of lard for the production of human milk fat substitute. *Eur. Food Res. Technol.* 2010, 230, 769–777.
- [15] Soumanou M. M., Pérignon M., Villeneuve P., Lipase-catalyzed interesterification reactions for human milk fat substitutes production: A review. *Eur. J. Lipid Sci. Technol.* 2013, 115, 270–285.
- [16] Sahin N., Akoh C. C., Karaali A., Human milk fat substitutes containing omega-3 fatty acids. *J. Agr. Food Chem.* 2006, 54, 3717–3722.
- [17] Sahin N., Akoh C. C., Karaali A., Lipase-catalyzed acidolysis of tripalmitin with hazelnut oil fatty acids and stearic acid to produce human milk fat substitutes. *J. Agric. Food Chem.* 2005, 53, 5779–5783.
- [18] Zou X. Q., Huang J. H., Jin Q. Z., Liu Y. F., et al., Lipase-catalyzed preparation of human milk fat substitutes from palm stearin in a solvent-free system. *J. Agr. Food Chem.* 2011, 59, 6055–6063.
- [19] Sørensen A. D. M., Xu X., Zhang L., Kristensen J. B., Jacobsen C., Human milk fat substitute from butterfat: Production by enzymatic interesterification and evaluation of oxidative stability. *J. Am. Oil Chem. Soc.* 2010, 87, 185–194.
- [20] Senanayake S. N., Shahidi F., Enzymatic incorporation of docosahexaenoic acid into borage oil. *J. Am. Oil Chem. Soc.* 1999, 76, 1009–1015.
- [21] Zou X. Q., Huang J. H., Jin Q. Z., Liu Y. F., et al., Preparation of human milk fat substitutes from palm stearin with arachidonic and docosahexaenoic acid: Combination of enzymatic and physical methods. *J. Agr. Food Chem.* 2012, 60, 9415–9423.
- [22] Luddy F., Barford R., Herb S., Magidman P., Riemenschneider R., Pancreatic lipase hydrolysis of triglycerides by a semimicro technique. *J. Am. Oil Chem. Soc.* 1964, 41, 693–696.
- [23] Zou X. Q., Huang J. H., Jin Q. Z., Guo Z., et al., Model for human milk fat substitute evaluation based on

- triacylglycerol composition profile. *J. Agr. Food Chem.* 2012, 61, 167–175.
- [24] Xu X., Production of specific-structured triacylglycerols by lipase-catalyzed reactions: A review. *Eur. J. Lipid Sci. Technol.* 2000, 102, 287–303.
- [25] Miller D. A., Prausnitz J. M., Blanch H. W., Kinetics of lipase-catalysed interesterification of triglycerides in cyclohexane. *Enzyme Microb. Tech.* 1991, 13, 98–103.
- [26] Xu X., Skands A. R. H., Høy C. E., Mu H., et al., Production of specific-structured lipids by enzymatic interesterification: Elucidation of acyl migration by response surface design. *J. Am. Oil Chem. Soc.* 1998, 75, 1179–1186.
- [27] Iyer P. V., Ananthanarayan L., Enzyme stability and stabilization-aqueous and non-aqueous environment. *Process Biochem.* 2008, 43, 1019–1032.
- [28] Xu X., Engineering of enzymatic reactions and reactors for lipid modification and synthesis. *Eur. J. Lipid Sci. Technol.* 2003, 105, 289–304.
- [29] Macrae A., Lipase-catalyzed interesterification of oils and fats. *J. Am. Oil Chem. Soc.* 1983, 60, 291–294.
- [30] Xu X., Mu H., Skands A., Høy C. E., Adler-Nissen J., Parameters affecting diacylglycerol formation during the production of specific-structured lipids by lipase-catalyzed interesterification. *J. Am. Oil Chem. Soc.* 1999, 76, 175–181.