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Lipase-catalyzed modification of lard to produce human milk fat substitutes

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Abstract

The objective of the present work was to modify lard into human milk fat substitutes (HMFS) by Lipozyme RM IM-catalyzed acidolysis. Lard and soybean fatty acids were esterified in a solvent-free system. The reaction substrates for HMFS production were specially chosen to mimic human milk fats. Factors such as temperature, time, water content, enzyme load, substrate ratio, and enzyme reusability were investigated. The relationships between initial incorporation rate (Inc/h) and temperature (T, K) were set up, based on the Arrhenius law for both linoleic and for linolenic acids. Scale-up trials were carried out to confirm the feasibility of enzymatic modification for the production of HMFS. The characteristics of the product, produced in the scale-up acidolysis under selected conditions (temperature 61 °C, water content 3.5%, lard:fatty acids 1/2.4 (mol/mol), Lipozyme RM IM load 13.7%, and time 1.0 h), were similar to the fat in Chinese mothers' milk. The results showed that it was possible to produce human milk fat substitutes from lard through enzymatic acidolysis with soybean fatty acids.

Keywords: Human milk fat substitutes; Lard; Acidolysis; Lipase; Lipozyme; Soybean oil

1. Introduction

Human milk is naturally the only source of food for infants in their early life. Lipids in human milk provide a major source of energy and essential structural components for the cell membranes of the newborn (Christie, 1995). Fatty acid compositions of lipids in human milk vary with such factors as diet, lactation stage, season, and individual conditions (Christie, 1995; Jensen, 1998; Clark & Hundrieser, 1993). However, a similar general structural pattern can be seen in the corresponding triacylglycerols (TGs) of human milks. At the sn-2 position of the TGs, 60–70% of fatty acids are palmitic acid (16:0). This structure is important because TGs are exposed as such to lipolytic enzymes and not as fatty acids (De Fouw, Kivits, Quinlan, & van Nielen, 1994; Lien, 1994; Lien, Boyle, Yuhas, Tomarelli, & Quinlan, 1997; Lucas et al. 1997; Summers, Fielding, Ilic, Quinlan, & Frayn, 1998). Structure is also one of the key factors controlling the products formed by the gastric lipase in the stomach and by pancreatic or bile salt-stimulated lipases in the small intestinal absorption (Jensen et al., 1995). Pancreatic lipase selectively hydrolyses the fatty acids at the sn-1 and sn-3 positions, yielding free fatty acids and monoacylglycerols (MGs). The 2-MGs are absorbed more efficiently than free palmitic acid, which formed insoluble soaps with calcium and magnesium (Forsyth, 1998). Innis, Dyer, and Nelson (1994) provided convincing evidence that palmitic acid was absorbed as sn-2 MG in breast-fed infants. Carnielli et al. (1996) and Kennedy et al. (1999) reported similar evidence.

On the other hand, many mothers have to depend on man-made formulas to feed their babies, for medical, metabolic, and economical reasons. The fatty acid composition and distribution of TGs have recently gained much attention in infant formulas. Human milk TGs are believed to be a model for the fat components in infant formulas (Jensen, 1998). To produce the major TGs of human milk fat, i.e.1,3-diunsaturated-2-satu-

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rated TGs, chemical approaches are usually impossible, due to the lack of positional specificity (Quinlan, Lockton, Irwin, & Lucas, 1995). Many scientists have proved that these structured lipids would be most effectively produced by enzymatic interesterification with sn-1,3 specific lipases (Akoh & Moussata, 1998; Gunstone, 1999; Quinlan & Chandler, 1992; Xu, Balchen, Høy, & Adler-Nissen, 1998; Xu, Mu, Høy, & Adler-Nissen, 1999).

Loders Croklaan (Unilever) has commercialized a human milk fat substitute (HMFS) product based on the enzyme process from palm stearin (Quinlan, Lockton, Irwin, & Lucas, 1995). Shimada et al. (2000) synthesized 1,3-arachidonoyl-2-palmitoyl-glycerols by acidolysis of palm stearin with arachidonic acid using the 1,3-specific *Rhizopus delemar* lipase. Suntory, Japan (Akimmoto, Yaguchi, & Fujikawa, 1999) patented a similar enzyme method to produce HMFS rich in polyunsaturated fatty acids. Lipase-catalyzed modification of butter oil was conducted to produce a fat resembling human milk fat in a laboratory solvent system (Christensen & Holmer, 1993). A "two-step" process was also introduced for the production of HMFS, in which alcoholysis was the first step and esterification was the second step (Schmid, Bornscheuer, Soumanou, McNeill, & Schmid, 1998). No reports have so far been published on the enzymatic production of HMFS from lard.

Many other concerns for the lipids in infant nutrition have been addressed in a recent book (Huang & Sinclair, 1998). In this work the objective is to produce HMFS by lipase-catalyzed acidolysis of lard with soybean fatty acids. The reaction substrates were specially chosen in order to mimic human milk fats. Reaction conditions, such as temperature, time, water content, enzyme load, substrate ratio, and enzyme reusability, were investigated. Scale-up trials were conducted to confirm the feasibility of enzymatic modification of lard for the production of HMFS.

2. Materials and methods

2.1. Materials

Soybean fatty acids were prepared by saponification of soybean oil, acidification, extraction, and drying (Jenkins, Kramer, Sauer, & Emmons, 1985). Pancreatic lipase was prepared from porcine pancreas according to the IUPAC method (Paquot & Hautfenne, 1987) (activity 1.96U; the lipase unit is defined as the amount of enzyme, which will liberate 10μ equivalents of acid per minute). Lipozyme RM IM, a commercial sn-1,3 specific lipase, in which *Rhizomucor miehei* lipase is immobilized on a microporous ion exchange resin, was donated by Novozymes A/S, Bagsvaerd, Denmark. Fatty acid methyl ester standards were purchased from Sigma (St. Louis, MO). All other reagents and solvents were of analytical grade.

2.2. Process methods

Ten grammes of reactants (lard and fatty acids) in a 50 ml conical flask were used for the Lipozyme RM IM-catalyzed acidolysis under 250 rpm magnetic stirring. Individual conditions are indicated in each figure. Water content in this text (calculated as wt.% on enzyme basis) included all sources (added water and water present in the lipase and substrates). The adjustment of water content was done by the direct addition of distilled water to the enzyme, conditioned overnight and measured by the IUPAC standard method (Paquot & Hautfenne, 1987) before reaction. Samples were withdrawn during the reaction and stored in the freezer before analysis.

2.3. Triacylglycerol isolation

Samples were isolated by thin-layer chromatography (TLC, silicic acid 60G, thickness 1 mm, 20 cm×20 cm) with petroleum ether/diethyl ether/acetic acid 80/20/2 (v/v/v) as developing solvent. The band containing triacylglycerols (TGs) was scraped off and extracted 3 times with hexane. The solvent was removed under a stream of N_2. The isolated TGs were used for the analysis of fatty acid compositions of both total and those from the sn-2 positions.

2.4. Methylation and GC analysis

Fatty acid (FA) methyl esters of the isolated TGs were prepared by saponification of TGs and esterification with methanol in the presence of boron trifluoride according to the IUPAC standard method (Paquot & Hautfenne, 1987). The methyl esters were analyzed by GC with a GC-9A chromatograph (Shimadzu, Kyoto, Japan) equipped with a 25 m fused-silica capillary column (0.33mm id) coated with PEG-20M (Shimadzu, Kyoto, Japan). A flame ionization detector was used. The injector and detector temperatures were 250 °C. Column oven was operated at 195 °C under isothermal control. The flow rate of the carrier gas (nitrogen) was 70 ml/min. Area percentages were recalculated into molar percentages according to response factors measured.

2.5. Pancreatic lipase hydrolysis

Pancreatic lipase hydrolysis was used to determine the FA composition at the sn-2 position of the isolated TGs. The analysis was conducted according to the IUPAC standard method (Paquot & Hautfenne, 1987).

The monoacylglycerols (sn-2 MGs), after pancreatic lipase hydrolysis, were separated on TLC plates (silicic acid 60G, thickness 1 mm, 20 cm×20 cm) with hexane/diethyl/formic acid (70/30/1, v/v/v) as the developing solvent. The developed plates were air-dried, sprayed with a 2 g/l solution of 2,7-dichlorofluorescein in 95% (v/v) ethanol, and visualized under ultraviolet light. The band containing sn-2 MGs was scraped off and transferred to the methylation flask. The MGs were converted into FA methyl esters by the boron trifluoride method (Paquot & Hautfenne, 1987). The FA methyl esters were analyzed by GC as described above.

2.6. Melting point determination

Melting point determination was carried out by the capillary tube method according to the AOCS official method Cc 1–25 (AOCS, 1998).

3. Results and discussion

3.1. Selection of substrate acyl donors

Fatty acid profiles of human milks change slightly with differences of maternal diet, length of gestation, duration of lactation and parity (Christie, 1995). Typical fatty acid composition and fatty acid distribution of human milk fat on western diets are presented in Table 1. It is obvious that most of the palmitic acid is located at the sn-2 position (68%). A typical fatty acid composition of milk fat in Chinese mothers is: C16:0 (18.8%), C18:0 (5.5%), C16:1 (3.4%), C18:1n-9 (28.0%), C18:2n-6 (21.0%), and C18:3n-3 (3.0%), which has more C18:2n-6 and C18:3n-3 and less trans fatty acids than the milk from western mothers (Ruan et al., 1995).

Table 1
Typical fatty acid composition (mol%) and distribution of human milk fat[a]

Fatty acid[b]	Total	sn-2	%sn-2[c]	sn-1,3[d]
C12:0	4.9	5.3	36.0	4.7
C14:0	6.6	11.2	57.0	4.3
C16:0	21.8	44.8	68.0	10.3
C18:0	8.0	1.2	5.0	11.4
C18:1n-9	33.9	9.2	9.0	46.3
C18:2n-6	13.2	7.1	18.0	16.3
C18:3n-3	1.2	–	–	–

[a] Values are derived from Lien et al. (1997).
[b] C12:0, lauric; C14:0, myristic; C16:0, palmitic; C18:0, stearic; C18:1n-9, oleic; C18:2n-6, linoleic; and C18:3n-3, linolenic acids.
[c] Indicates the percentage of the fatty acids esterified at the sn-2 position, calculated as [sn-2 fatty acids×100% /(3×total fatty acids)].
[d] Indicates fatty acid composition at the sn-1,3 positions, calculated as [3×Total−(sn-2)]/2.

The development of enzyme technology, particularly the commercial production and applications of sn-1,3 specific lipases, makes it possible to produce HMFS from vegetable or animal oils that better mimic the fatty acid and TG compositions of human milk fats. The following reaction scheme is often used:

$$\text{TGs} + \text{FFAs (acyl donors)} \xrightarrow{sn-1,3\ specific\ lipases} \text{HMFS}$$

With the above scheme, the TGs should contain palmitic acid primarily at the sn-2 position. Palm stearin, a fraction of palm oil with a high content of tripalmitin, has been selected as the starting TGs by Loders Croklaan and many studies for the enzymatic production of HMFS. However, lard is a fat available in large quantities, which has a TG structure similar to that found in human milk fats although it is not acceptable to certain groups of people (Jensen, 1998). Therefore, lard, which is acceptable and cheap in the Chinese market, was chosen in this study.

Compared with human milk fat, especially with that from Chinese mothers (Ruan et al., 1995), lard contains less essential fatty acids, i.e. linoleic and linolenic acids (Table 2). In most human milk fats, the ratio of linoleic acid to linolenic acid (LA/ALA) is generally between 5 and 15, but LA/ALA in lard is around 33 and this is markedly different from that of human milks. From this consideration, acyl donors should be rich in both linoleic and linolenic acids and also have a proper ratio of LA/ALA to meet the requirements for infants.

In enzymatic acidolysis with sn-1,3 specific lipases, the percentages of certain fatty acid at the sn-1,3 position (sn-1,3%) and in total (Total%) fatty acid compositions in the product TGs can be estimated by the following equations:

$$\text{sn}-1,3(\%) = \frac{(2 - S_r D_a)R_F + A_F S_r D_a}{2} \quad (1)$$

$$\text{Total}(\%) = \frac{(2 - S_r D_a)R_F + A_F S_r D_a + (sn-2)}{3} \quad (2)$$

$$A_F(\%) = \frac{3\times\text{Total} - (2 - S_r D_a)R_F - (\text{sn-2})}{S_r D_a} \quad (3)$$

where S_r, D_a, R_F, A_F, and sn-2 are substrate mole ratios (acyl donors:lard), degree of conversion (incorporated content of the fatty acid/initial content of the fatty acid in acyl donors), the amount of the fatty acid at the sn-1,3 positions of lard (mol%), the amount of the fatty acid in acyl donors (mol%), and the amount of the fatty acid at the sn-2 position (mol%), respectively.

Conversion degree (D_a) at equilibrium is about 25–75% when S_r is correspondingly 6–2 in acidolysis reactions (Xu, 2000a). If fatty acid compositions of milk fats from Chinese mothers are considered as the target, the

Table 2
Typical fatty acid composition (mol%) and distribution of lard[a]

Fatty acid[b]	Total	sn-2	%sn-2[c]	sn-1,3[d]
C12:0	0.1	0.2	83.3	0.0
C14:0	1.0	2.4	76.6	0.4
C16:0	23.9	67.3	93.8	2.2
C18:0	15.4	8.3	18.0	19.0
C18:1n-9	41.6	12.8	10.0	56.0
C18:2n-6	13.2	5.7	14.3	17.0
C18:3n-3	0.4	0.4	33.3	0.4

[a] Values are derived from Lien et al. (1997).
[b] C12:0, lauric; C14:0, myristic; C16:0, palmitic; C18:0, stearic; C18:1n-9, oleic; C18:2n-6, linoleic; and C18:3n-3, linolenic acids.
[c] Indicates the percentage of the fatty acids esterified at the sn-2 position, calculated as [sn-2 fatty acids×100% /(3×total fatty acids)].
[d] Indicates fatty acid composition at the sn-1,3 positions, calculated as [3×Total−(sn-2)]/2.

contents of oleic, linoleic and linolenic acids in acyl donors should be about 22, 52 and 13% (mol/mol), respectively, according to Eq. (3), supposing D_a is 30% and S_r is 2. After evaluating fatty acid compositions of various oils and fats, it was found that the fatty acids of soybean oil were suitable for use as the acyl donors in this reaction. Fatty acid composition in the soybean oil from northeast China is (mol%): C16:0 (11.9), C18:0 (2.4), C18:1n-9 (21.1), C18:2n-6 (51.3), C18:3n-3 (12.9), and others (0.4) and the fatty acid mixture was used in the present study.

3.2. Effect of temperature

An increase of reaction temperature usually results in an acceleration effect, according to the Arrhenius law, during reactions catalyzed by enzymes. High temperature favours higher yields for endothermic reactions due to the shift of thermodynamic equilibrium. At elevated temperatures, operation is also easy, since higher temperature increases solubility of reagents and decreases the viscosity of solutions (Martinek, 1993). In the present reaction system, temperatures from 50 to 90 °C were taken into consideration in order to keep the lard in the liquid state. As the temperature increased, the time to reach the reaction terminal, defined as 20 mol% incorporation of C18:2n-6 and 3 mol% C18:3n-3 in the produced TGs, decreased from 5 h at 50 °C to 2.4 h at 90 °C (Fig. 1). The time-course variations of C18:2n-6 and C18:3n-3 incorporation were also monitored at 50, 60, 70, 80, and 90 °C, respectively (Fig. 2). As can be seen, the time courses of C18:2n-6 and C18:3n-3 were different. The explanation is complicated by the differences of their contents in both lard and acyl donors. However, higher temperature favoured more migration as well (Fig. 3). In the figure, migration is defined as the percentage change of palmitic acid at the sn-2 position. Xu (2000a) also reported that increased temperatures produced more by-products due to the occurrence of migration. Furthermore, higher temperature will lead to higher lipase deactivation rates (Martinek, 1993). A compromise between incorporation, migration, and lipase deactivation has to be made to choose the right reaction temperature.

Based on the Arrhenius law, the initial enzyme reaction rate, defined as the initial incorporation rate (Inc/h) calculated during the first 30 min, can be described as follows:

$$\ln(\text{Inc/h}) = \ln A - \frac{E}{RT} \tag{4}$$

where A = the Arrhenius constant, E = the activation energy, R = the gas constant, and T = temperature (K). Based on results from Fig. 2, the initial incorporation rates (Inc/h) can be calculated at different temperatures. Therefore, good linear relationships between ln(Inc/h) and $1/T$ for C18:2n-6 and C18:3n-3 were obtained with

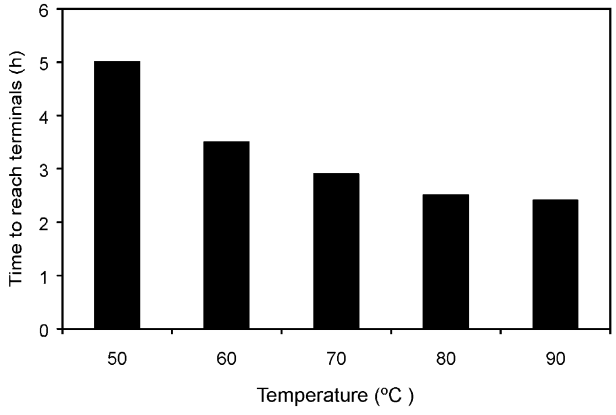

Fig. 1. Effect of temperature on reaction terminals in the acidolysis of lard with soybean fatty acids. Substrate mole ratio (lard:fatty acids), 1:2; water content (based on lipase), 3.5 wt.%; Lipozyme RM IM load (based on substrates) 10 wt.%.

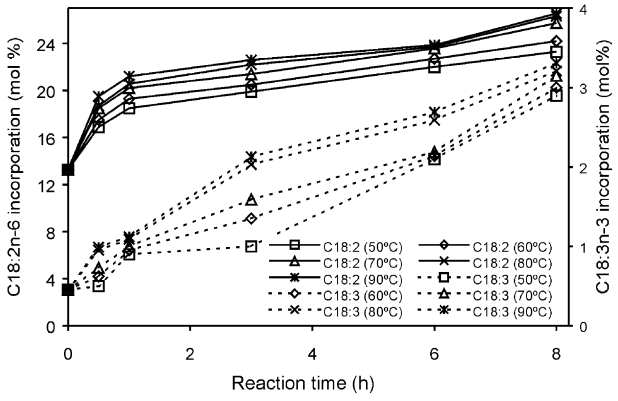

Fig. 2. Time courses of incorporation of linoleic (C18:2n-6) and linolenic (C18:3n-3) acids during the acidolysis of lard with soybean fatty acids at different temperatures. Conditions see Fig. 1.

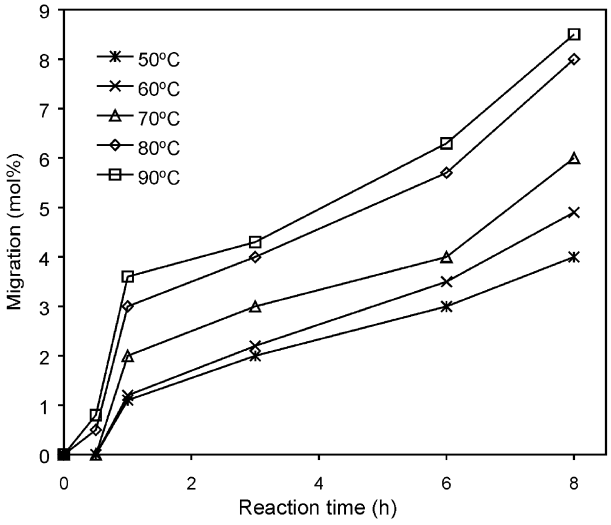

Fig. 3. Effect of temperature on migration during the acidolysis of lard with soybean fatty acids. Conditions see Fig. 1.

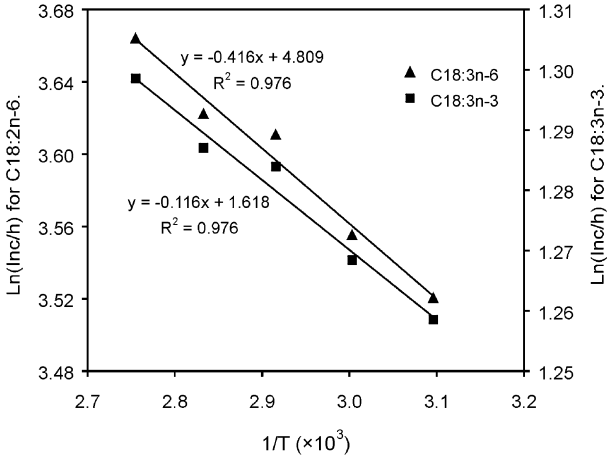

Fig. 4. Relationship between the initial rate of acyl incorporation and temperature (T, K) during the acidolysis of lard with soybean fatty acids. Conditions see Fig. 1.

satisfactory correlations (Fig. 4). Thus Eq. (4) can be written into Eq. (5) for C18:2n-6 and Eq. (6) for C18:3n-3 in the Lipozyme RM IM-catalyzed acidolysis of lard with soybean fatty acids in the present reaction system.

$$\ln(\mathrm{Inc/h}) = 4.809 - \frac{416}{T} \tag{5}$$

$$\ln(\mathrm{Inc/h}) = 1.618 - \frac{116}{T} \tag{6}$$

3.3. Effect of substrate ratio

The TG compositions of the products in the enzymatic acidolysis reaction depend on the substrate ratios (acyl donors/TGs) after reaction equilibrium has been achieved. Provided that the lipase has the same selectivity towards all fatty acids and also the same regiospecificity towards sn-1 and sn-3 positions, the equilibrium or maximum incorporation of all acyl donors can be theoretically calculated by the following equation if no side reactions occur (Xu, 2000b):

$$\mathrm{Inc}_{\max} = \frac{66.67}{1 + 2/S_{\mathrm{r}}} \tag{7}$$

where $\mathrm{Inc}_{\max}$ is the equilibrium acyl incorporation and *S*r is the substrate mole ratio. In this study, the effect of the amount of acyl donors in the substrate on the incorporation of C18:2n-6 and C18:3n-3 was investigated. When S_{r} was increased from 1 to 8, the C18:2n-6 and C18:3n-3 incorporation also increased (Fig. 5). However, the differences between S_{r} 6 and 8 were not very significant in the incorporation courses. These results are also in agreement with Eq. (7) with respect to maximum incorporation. Higher Sr would certainly move the reaction equilibrium to the product side and improve the acyl incorporation. The choice of substrate mole ratio is also related to the downstream processing cost and the associated difficulties of separating free fatty acids or acyl donors by evaporation and/or distillation. Therefore, a reasonable amount of substrate should be selected, based on all these considerations.

3.4. Effect of lipase load

Enzyme content is related to reaction rate. Higher enzyme load will accelerate the reaction rate and improve the incorporation of acyl donors in the acidolysis reaction. At the same time, the increase of enzyme load will also increase acyl migration (Xu, 2000a, 2000b). The C18:2n-6 and C18:3n-3 incorporation increased with increasing lipase content in the present study, suggesting that the reaction velocity was related to the content of Lipozyme RM IM catalyst (Fig. 6). However, the acidolysis extent, above 15% enzyme load, only slightly changed, especially for C18:2n-6. Enzyme load, in general, influenced the incorporation of C18:n-3 more strongly than C18:2n-6. This will definitely affect the final fatty acid profile of the products. Therefore, the correct enzyme load is also important in batch systems for shaping the fatty acid profiles of the products.

3.5. Effect of water content

In enzymatic acidolysis, two-step reactions, i.e. hydrolysis and esterification, usually take place. The hydrolysis is usually considered as the rate-limiting step. Obviously, water acts as a reactant in the hydrolysis step and a product in the esterification step, and therefore it plays an important role in the lipase-catalyzed acidolysis. At certain ranges, an increase of water content

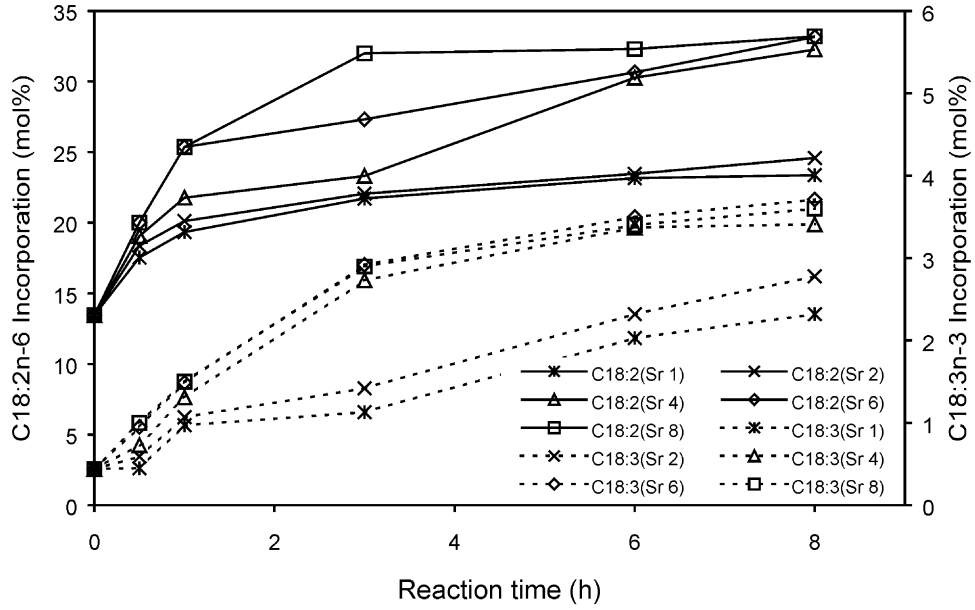

Fig. 5. Time courses of incorporation of linoleic (C18:2n-6) and linolenic (C18:3n-3) acids during the acidolysis of lard with soybean fatty acids at different substrate mole ratio (fatty acids:lard).Conditions: temperature 60 °C, water content (based on lipase) 3.5 wt.%; and Lipozyme RM IM load (based on substrates) 10 wt.%.

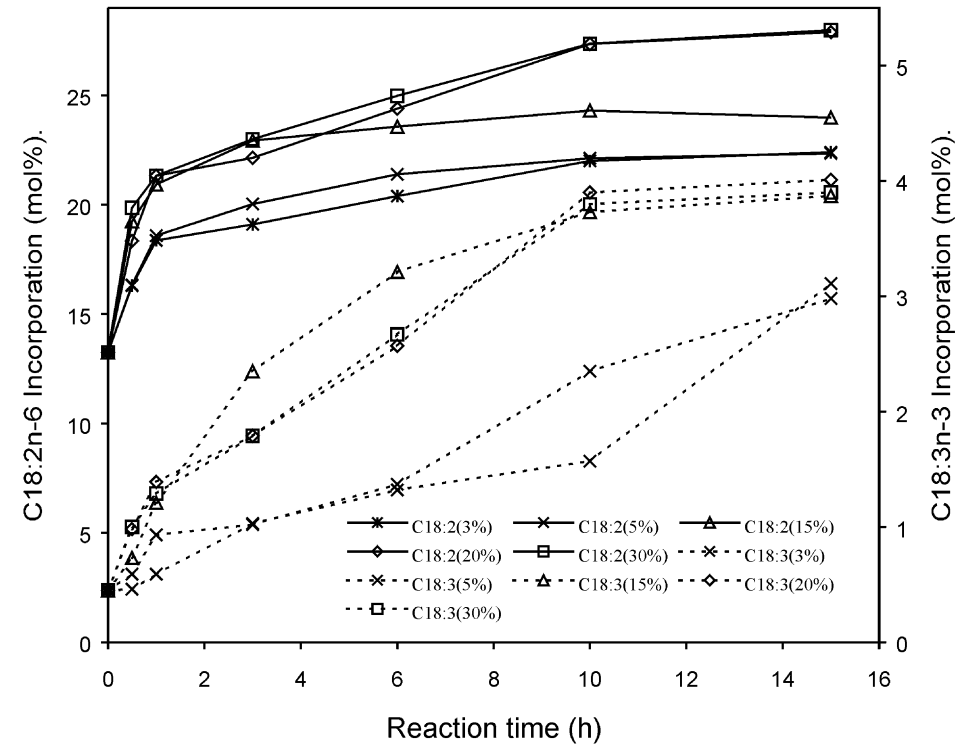

Fig. 6. Time courses of incorporation of linoleic (C18:2n-6) and linolenic (C18:3n-3) acids during the acidolysis of lard with soybean fatty acids at different enzyme load (based on total substrates). Conditions: substrate mole ratio (fatty acids:lard) 2, temperature 60 °C, water content (based on lipase) 3.5 wt.%.

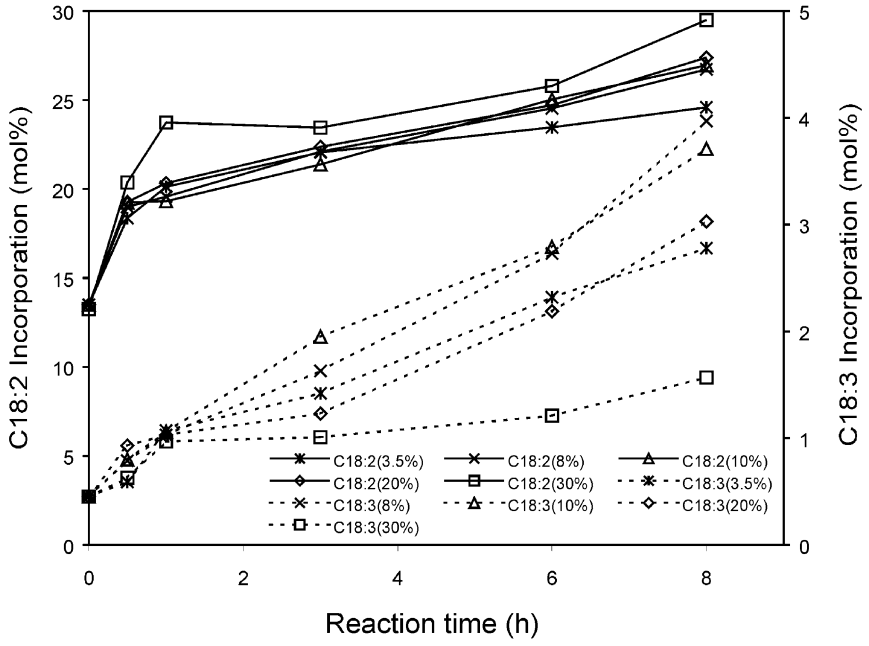

Fig. 7. Time courses of incorporation of linoleic (C18:2n-6) and linolenic (C18:3n-3) acids during the acidolysis of lard with soybean fatty acids at different water contents. Conditions: substrate mole ratio (fatty acids:lard) 2, temperature 60 °C, and Lipozyme RM IM load (based on substrates) 10 wt.%.

increases the initial activity of lipases. Too much water, however, does not enhance the whole reaction or the final product yield; instead it leads to a decrease in lipase activity and an increase in by-product formation. Therefore, optimal water content should be determined for a given reaction (Xu, 2000a, 2000b). In the acidolysis of lard with soybean fatty acids, C18:2n-6 incorporation increased with the increase in water content in general, but the increase was not significant (Fig. 7). For C18:3n-3 incorporation, 8–10% water content was optimal, and the C18:3n-3 incorporation was lowest at 30% water content (Fig. 7).

3.6. Scale-up trial

To verify the feasibility of the scaled-up acidolysis between lard and soybean fatty acids, a 100 g batch reaction (10 times scaling-up) was carried out under the

Table 3
Fatty acid composition (mol%) and other characteristics of human milk fat substitutes

Fatty acid[a]	Total	sn-2	sn-1,3
C14:0	1.3	2.0	1.0
C16:0	29.0	71.1	8.0
C18:0	8.9	5.6	10.6
C18:1n-9	34.7	15.3	44.4
C18:2n-6	23.7	4.1	33.5
C18:3n-3	2.3	05	3.2
C18:2n-6/C18:3n-3		10.5	
Melting point (°C)		32–35	

[a] C12:0, lauric; C14:0, myristic; C16:0, palmitic; C18:0, stearic; C18:1n-9, oleic; C18:2n-6, linoleic; and C18:3n-3, linolenic acids.

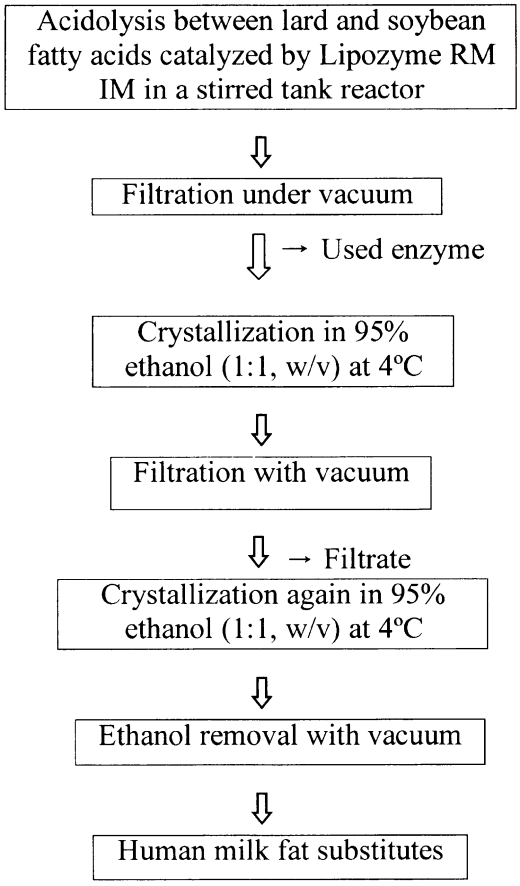

Fig. 8. Scale-up process for the enzymatic modification of lard to produce human milk fat substitutes. Reaction conditions: temperature 61 °C, water content (based on lipase) 3.5 wt.%, lard:fatty acids 1/2.4 (mol/mol), Lipozyme RM IM load 13.7 wt.% (based on substrates) and reaction time 1.0 h.

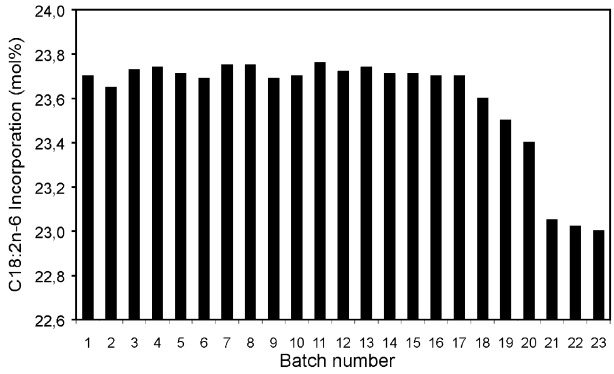

Fig. 9. Reusability of Lipozyme RM IM in the acidolysis of lard with soybean fatty acids. Reaction conditions see Fig. 8.

following conditions: temperature 61 °C, water content 3.5%, lard:fatty acids 1/2.4 (mol/mol), Lipozyme RM IM load 13.7% (based on the total substrate), and time 1.0 h. The whole process is depicted in Fig. 8. The results of the scale-up acidolysis are shown in Table 3. The characteristics of the product are similar to those of milk fats from Chinese mothers. The melting point (32–35 °C) and the ratio of LA/ALA (10.5) resemble those of natural human milk fats (Forsyth, 1998; Quinlan & Chandler, 1992).

3.7. Reusability of Lipozyme RM IM

To investigate the reusability of Lipozyme RM IM in the acidolysis of lard with soybean fatty acids, selected reaction conditions were used to test the stability of the lipase. The activity was expressed as the incorporation of C18:2n-6 in the produced TGs. After each batch, the lipase was filtered and recovered. Twenty-four batch cycles were conducted. The activity of Lipozyme RM IM (C18:2n-6 incorporation) was stable during the first 19 batches (Fig. 9). Lipozyme RM IM could be reused 23 times without significant loss of activity with no water re-adjustment.

4. Conclusion

Lipozyme RM IM-catalyzed acidolysis of lard with fatty acids of soybean oil could be used to produce HMFS in a solvent-free reaction system. The characteristics of the products are suitable for infant formula with respect to fatty acid composition, fatty acid distribution in TGs, and melting properties. The results suggest that it is practical to produce HMFS from lard by enzymatic acidolysis.

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