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Preparation and Characterization of 1,3-Dioleoyl-2-palmitoylglycerol

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ABSTRACT: 1,3-Dioleoyl-2-palmitoylglycerol, an important triacylglycerol in infant formulas, was effectively enriched by a two-step process: (a) dry fractionation of leaf lard and (b) enzymatic acidolysis of the fractionated leaf lard. In step a, the 1,3-dioleoyl-2-palmitoylglycerol content was increased from 16.77 to 30.73% after programmed temperature treatment of the leaf lard at 60 °C for 20 min followed by 34 °C for 10 h. In step b, 43.72% of the 1,3-dioleoyl-2-palmitoylglycerol content was obtained at the optimal conditions of enzymatic acidolysis: a substrate molar ratio of 1:4 (the fractionated leaf lard/camellia oil fatty acids), 6% (w/w) of enzyme loading, and 6 h of reaction time at 45 °C. On the basis of gas chromatography determination and "deducting score" principle, a model was properly established for characterizing the quality of triacylglycerols enriched with 1,3-dioleoyl-2-palmitoylglycerol. This approach would be a valuable contribution in structured lipids industries because only gas chromatography determination was involved.

KEYWORDS: 1,3-dioleoyl-2-palmitoylglycerol, quality assessment, leaf lard, enzymatic acidolysis, fractionation

INTRODUCTION

The nutritional value of triacylglycerols (TAGs) depends not only on the fatty acid composition but also on the positional distribution of the acyl groups along the glycerol backbone. Human milk fat (HMF) provides >50% of the dietary energy requirements for the infant.[1] The major saturated fatty acid in HMF is palmitic acid (C16:0), which is primarily located at the sn-2 position (>70%) of glycerol backbone, whereas the predominant unsaturated fatty acid, oleic acid (C18:1n-9c), occupies the sn-1,3 positions.[2,3] Studies have demonstrated that such a chemical structure helps the simultaneous absorption of fatty acids in the gut lumen of the infant and the loss of calcium through the feces.[4,5] In contrast with HMF, fats in most infant formulas containing C16:0 mostly at the extreme positions are hydrolyzed by pancreatic lipase, yielding free C16:0, which would generate poorly absorbed insoluble calcium soaps in the intestine. To achieve high quality of human milk fat substitutes in infant formulas, there is great interest in the synthesis of 1,3-dioleoyl-2-palmitoylglycerol (OPO) recently.

Numerous investigations have been focused on the production of TAGs enriched with OPO from vegetable oils. The production of TAGs with high content of OPO is generally achieved by acidolysis of tripalmitin and oleic acid using 1,3-specific lipases.[6−9] However, some drawbacks are put forward. First, large-scale application of tripalmitin is expensive in the market. Second, contrasting with HMF, the percentage of total C16:0 esterified at the sn-2 position and that of total C18:1n-9c at the sn-1,3 positions of the products, using tripalmitin as feedstock to produce OPO in one step, were rather undesirable.[6,7] These are mainly because the C16:0 is mostly located at the sn-1,3 positions and C18:1n-9c is principally esterified at the sn-2 position in vegetable oils. As a result, it is difficult to reach product quality standard and results in low economic benefit. Finally, the incorporation of C18:1n-9c was more interesting, but the percentage of C16:0 esterified at the sn-2 position was rarely discussed during acidolysis process. This is because the degree of acyl migration is another crucial parameter that might affect the quality of structured lipids.[10] In addition, different fatty acid composition and distribution of products are presented with gas chromatography (GC) determination, which makes it difficlt to evaluate the overall quality of TAGs enriched with OPO. As discussed in a previous publication,[2] the "deducting score" principle is proved to be considerably suitable for assessing the quality of structured lipids.

There are also other routes adopting multistep reactions that have appeared in the literature for the synthesis of OPO.[11−13] Chen et al.[11] synthesized OPO by a three-step enzymatic process: (a) fractionation of palm oil to obtain C18:1n-9c and C16:0, which was further transformed into ethyl palmitate, (b) glycerolysis of ethyl palmitate with glycerol catalyzed by Novozym 435 for the synthesis of tripalmitin, and (c) acidolysis of tripalmitin with C18:1n-9c catalyzed by lipase IM 60 to finally obtain OPO. However, this method was carried out in the organic solvent, which is not possible from the view of infant food safety. Besides, the use of two lipases will not facilitate any industrial application.

Alternatively, lard can be used as a substrate to obtain human milk fat substitutes.[14,15] Lard has a natural advantage in obtaining TAG products enriched with OPO because the main chemical structure of TAGs in lard is similar to that of HMF. Besides, lard is an inexpensive coproduct of the pork-packing industry and can be supplied in large quantities. A large amount of lard is significantly oversupplied every year because of its high content of saturated fatty acids and cholesterols. Thus, great attention should be paid to taking advantage of this low-cost and renewable resource to develop products with high added value.

The goals of this work were to effectively enrich the content of OPO in TAGs and to establish a simple and effective model for characterization of OPO. The preparation of OPO was carried out by a two-step process: fractionation of leaf lard and enzymatic acidolysis of the fractionated leaf lard. On the basis of the "deducting

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score" principle, a model with GC data was established to comprehensively characterize the quality of product enriched with OPO. This approach would be expected to contribute to the structured lipids industries because only GC analysis was involved.

MATERIALS AND METHODS

Materials. Camellia oil fatty acids were prepared according to the method described in the literature.[16] Porcine pancreatic lipase (type II, crude) was purchased from Sigma Chemical Co. (St. Louis, MO). Immobilized 1,3-specific lipase, Lipozyme RM IM, was purchased from Novo Nordisk A/S, Bagsvaerd, Denmark. A standard mixture with 37 fatty acid methyl esters (FAMEs) was purchased from Sigma. Leaf lard was bought from a local market. All other reagents and solvents used for analysis were of chromatographic or analytical grade.

Fractionation of Leaf Lard. Fractionation was carried out on 100 g of leaf lard in a 250 mL beaker with stirrer. The container was placed in a water bath, where the temperature control was programmed. The leaf lard was heated at 60 °C for 20 min, then cooled to 34 °C, and held for 10 h. The 34 °C liquid fractionated leaf lard (named 34L-leaf lard) was used in the subsequent enzymatic acidolysis reactions.

Enzymatic Acidolysis. The 34L-leaf lard (1.8 g) was mixed with camellia oil fatty acids (substrate molar ratios of 1:2.5, 1:4, and 1:5.5, 34L-leaf lard/camellia oil fatty acids) in a 25 mL conical flask. The mixture was treated with Lipozyme RM IM (6% of the total substrates in weight) and was incubated at 35, 45, and 55 °C, respectively, in an orbital shaking air bath at 200 rpm for 12 h. One milliliter of reaction mixture was sampled at reaction times of 3, 6, and 12 h.

Analysis of Products. The enzyme and moisture were removed from the reaction mixture by passing through an anhydrous sodium sulfate column. The reaction products were applied to thin layer chromatography (TLC) plates (10 × 20 cm) coated with silica gel G. The developing solvent was petroleum ether/ethyl ether/acetic acid (80:20:1, v/v/v). The bands were sprayed with 0.2% 2,7-dichlorofluorescein in methanol and visualized under ultraviolet (UV) light. The TAGs band was scraped off for the analysis of fatty acid composition and distribution.

Fatty Acid Composition of TAGs Enriched with OPO. Small amounts of scraped TAGs were methylated to FAMEs following the method of ISO 5509:2000(E).[2] The FAMEs were analyzed on an Agilent 7890A GC equipped with a capillary column CP-Sil 88 (60 m × 0.25 mm × 0.2 μm, DIKMA Inc.) and a flame ionization detector. The temperatures of the injector and detector were 250 and 300 °C, respectively. The column oven was initially held at 140 °C for 5 min and was then programmed to 220 °C at a rate of 4 °C/min and was held isothermally for 15 min. Nitrogen was used as the carrier gas at a head pressure of 0.5 MPa with a flow rate of 1.1 mL/min. The correction factor values of FAMEs were used to obtain the relative content of FAMEs expressed as molar percent.

Fatty Acid Composition at the sn-2 Position Analysis. The TAGs enriched with OPO were scraped from the TLC plate as described earlier and were applied for the sn-2 positional analysis using the method described by Sahin et al.[7] The 2-monoacyglycerol (2-MAG) band was scraped into a screw-capped test tube and was extracted with 2 mL of ethyl ether. Excess solvent was removed under nitrogen, and then 2-MAG fractions were methylated for GC analysis. The migration rate (MR) of C16:0 was defined as follows: MR = (%sn-2C16:0 at the initial reaction time) − (%sn-2C16:0 at different reaction time periods), where, %sn-2C16:0 represents the percentage of C16:0 esterified at the sn-2 position and it was calculated as

$$\%\text{sn-2C16:0} = \frac{\text{content of C16:0 at the sn-2 position}}{\text{content of C16:0 in the total fatty acids} \times 3} \times 100 \quad (1)$$

Reversed-Phase HPLC Analysis of TAGs. TAG fractions were separated by a serial liquid chromatograph (Waters Tech.) equipped with an XbridgeTM C18 column (250 mm × 4.6 mm, 5 μm particle size, Waters) and an evaporative light scattering detector (ELSD). The sample (5 mg) was dissolved in 1 mL of hexane, and acetonitrile and 2-propanol were used in gradient elution. Sixty-five percent of the initial elution was acetonitrile, and then the concentration of acetonitrile was decreased linearly with a gradient from 65 to 60% in the first 10 min and from 60 to 55% over the next 40 min. After completion of the chromatograph elution, the mobile phase was set to its initial concentration within 5 min. The flow rate was 0.8 mL/min, and the injection volume was 20 μL.

The peak identification of a single TAG was coupled with mass spectrometry (MS). The following MS conditions were used: nebulizer gas, N_2; P, 0.14 MPa; nebulizer gas flow rate, 10 mL/min; atmospheric pressure chemical ionization (APCI) mode, positive; APCI temperature, 350 °C; Q array (quadrupole array), scan; mass range, m/z 50−1500.

Assessment of the Quality of TAGs Enriched with OPO. Structured lipids have specific chemical structures that contribute to their special assimilated and nutritional functions. The chemical structure could be determined by two equally important elements: total fatty acid composition and sn-2 positional fatty acid composition. From the aspects of absorption and nutrition, evaluation indices, the percentage of C16:0 esterified at the sn-2 position (index I), and the content of C18:1n-9c in total fatty acids (index II) should be discussed to assess the quality of production with a high content of OPO. A model was established over a "deducting score" principle to evaluate the quality of TAGs enriched with OPO, and the model was expressed by following equations:

$$G = G_{\%\text{sn-2C16:0}} + G_{\text{C18:1n-9c}} \quad (2)$$

$$G_{\%\text{sn-2C16:0}} = 50 - E_{\%\text{sn-2C16:0}} \quad (3)$$

$$G_{\text{C18:1n-9c}} = 50 - E_{\text{C18:1n-9c}} \quad (4)$$

It was assumed that the maximum score for G (the quality of TAGs enriched with OPO) is 100 and 50 for each index ($G_{\%\text{sn-2C16:0}}$ and $G_{\text{C18:1n-9c}}$). $E_{\%\text{sn-2C16:0}}$ and $E_{\text{C18:1n-9c}}$ were the corresponding deducting scores calculated from the following equations:

$$E_{\%\text{sn-2C16:0}} = 50 \times (C_{\%\text{sn-2C16:0}} \times D_{\%\text{sn-2C16:0}}) \quad (5)$$

$$E_{\text{C18:1n-9c}} = 50 \times (C_{\text{C18:1n-9c}} \times D_{\text{C18:1n-9c}}) \quad (6)$$

$D_{\%\text{sn-2C16:0}}$ and $D_{\text{C18:1n-9c}}$ were the weights of indices. It was assumed that $D_{\%\text{sn-2C16:0}} = D_{\text{C18:1n-9c}} = 1$. $C_{\%\text{sn-2C16:0}}$ and $C_{\text{C18:1n-9c}}$ were floating rates for indices I and II and were calculated by the following equations:

$$C_{\%\text{sn-2C16:0}} = \frac{|B_{\%\text{sn-2C16:0}} - A_{\%\text{sn-2C16:0}}|}{A_{\%\text{sn-2C16:0}}} \quad (7)$$

$$C_{\text{C18:1n-9c}} = \frac{|B_{\text{C18:1n-9c}} - A_{\text{C18:1n-9c}}|}{A_{\text{C18:1n-9c}}} \quad (8)$$

$B_{\%\text{sn-2C16:0}}$ is the percentage of C16:0 distributed at the sn-2 position of TAGs, and the values of different samples were calculated by eq 1. $B_{\text{C18:1n-9c}}$, the actual measured value of different samples, was the content of C18:1n-9c in total fatty acids. $A_{\%\text{sn-2C16:0}}$ and $A_{\text{C18:1n-9c}}$ were theoretical values (100 and 66.67%, respectively) in pure OPO.

The rationality and accuracy of the established model was verified using different fats and oils such as different synthesized OPO products, leaf lard, and vegetable oils and also by the HPLC analysis results obtained from two-step preparation of OPO. The total score ($G = G_{\%\text{sn-2C16:0}} + G_{\text{C18:1n-9c}}$) was used to systematically evaluate the quality of TAGs enriched with OPO.

Table 1. Total Fatty Acid and sn-2 Positional Fatty Acid Composition of Leaf Lard and 34L-Leaf Lard (mol%)

	leaf lard			34L-leaf lard		
fatty acid[a]	total fatty acid profile	sn-2 profile	RP[b]	total fatty acid profile	sn-2 profile	RP
C14:0	1.79	4.93	91.79	1.89	4.01	70.72
C16:0	27.62	71.69	86.53	27.13	66.51	81.72
C16:1	1.36	2.15	52.73	2.56	3.25	42.32
C18:0	16.72	3.70	7.39	13.17	4.65	11.77
C18:1n-9c	29.47	7.59	8.59	41.18	14.90	12.06
C18:2n-6c	19.46	6.92	91.79	11.48	4.36	70.72

[a] Fatty acid abbreviations: C14:0, myristic acid; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1n-9c, oleic acid; C18:2n-6c, linoleic acid. [b] Relative percentage of each fatty acid esterified at the sn-2 position, calculated by eq 1.

RESULTS AND DISCUSSION

Fractionation of Leaf Lard (First Step of Preparation of OPO). Lard is readily available at fairly low prices. However, its inherent defects such as high content of stearin acid and high melting point limit its utilization and increase the operational difficulty. The high melting point of leaf lard makes it difficult to effectively carry out reactions without solvent, especially in enzymatic processing of structured lipids.

To enrich the content of OPO, a liquid fraction (34L-leaf lard) was separated from leaf lard by dry fractionation at a crystallization temperature of 34 °C. The fatty acid composition and distribution of leaf lard and 34L-leaf lard are presented in Table 1. The content of C18:1n-9c approximately increased 11.7% in 34L-leaf lard when compared with that in leaf lard (29.47%). The percentage of total C16:0 distributed at the sn-2 position in 34L-leaf lard was reduced to 81.72%, which was lower than that of total C16:0 distributed at the sn-2 position (86.53%) in leaf lard. The contents of stearin acid and linoleic acid were 13.17 and 11.48% of the total fatty acids in 34L-leaf lard, whereas those of these acids in leaf lard were higher (Table 1). The results obtained from GC determination were in good agreement with HPLC-ELSD and APCI-MS analysis of TAG species (data not shown). After fractionation, large quantities of high-melting compounds (PSO, PPS, and PPS) were removed, and unsaturated TAGs (mostly OPO, from 16.77 to 30.73%) were enriched in 34L-leaf lard. Fractionation is an economical and effective method to considerably enrich the content of target compound of OPO in 34L-leaf lard.

Melting profiles of leaf lard and 34L-leaf lard were determined by differential scanning calorimetry (DSC, model SDTQ600). In contrast with leaf lard, 34L-leaf lard had a lower melting point. The main peak observed for 34L-leaf lard was at a melting temperature of 27.0 °C, whereas those for leaf lard were at melting temperatures of 31.4, 36.5, and 46.3 °C. Karabulut et al.[17] reported that the substrate with low viscosity could be easily accessed to the active site of the lipase during a low-temperature enzymatic reaction. Meanwhile, acyl migration was less sensitive below 50 °C in the 1,3-specific lipase-catalyzed system.[18] Thus, the fractionation process also facilitates further enzymatic enrichment of OPO in 34L-leaf lard.

Enzymatic Acidolysis (Second Step of Preparation of OPO). *Effect of Substrate Molar Ratio.* The effect of substrate molar ratio on the incorporation of C18:1n-9c is shown in Figure 1. The increase of substrate molar ratio favored the incorporation of C18:1n-9c as reported by several researchers.[7,19] At a reaction time of 6 h, the reaction equilibrium was almost achieved, and the

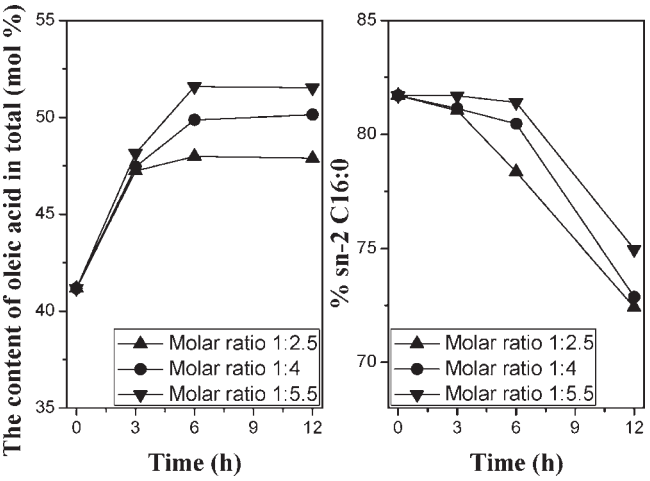

Figure 1. Effect of substrate molar ratio (34L-leaf lard/camellia oil fatty acids) on the incorporation of C18:1n-9c and on %sn-2C16:0. The reaction mixture contained substrate molar ratios of 1:2.5, 1:4, and 1:5.5 (34L-leaf lard was kept constant at 1.8 g) and 6% (total weight of substrates) of Lipozyme RM IM. The reaction mixture was incubated at 45 °C for 12 h in an orbital shaking air bath at 200 rpm.

incorporation of C18:1n-9c was 49.87 and 51.59% at substrate molar ratios of 1:4 and 1:5.5, respectively. Although the highest incorporation of C18:1n-9c (51.59%) was achieved at a substrate molar ratio of 1:5.5 at the reaction time of 6 h, the high content of free fatty acids in the acidolysis reaction would complicate the downstream processing and reduce the economic benefits. From Figure 1, the percentage of C16:0 located at the sn-2 position (%sn-2C16:0) was reduced when the substrate molar ratio was increased from 1:2.5 to 1:5.5. At the reaction time of 6 h, the migration rates of C16:0 from the sn-2 position to the sn-1,3 positions at different substrate molar ratios were determined to be 3.35, 1.25, and 0.30%, respectively. It could be seen that a high concentration of camellia oil fatty acids could lower the migration rate of C16:0 from the sn-2 position to the sn-1,3 positions, and the reason might be related with the high content of oleic acid. It has been reported that fatty acids with different chain lengths had different migration rates,[20] and oleic acid has the lowest migration among the C18 fatty acids.[18] On the basis of the above results, the substrate molar ratio of 1:4 was selected for further study.

Effect of Temperature. The effect of temperature on the content of C18:1n-9c and %sn-2C16:0 (the percentage of C16:0 esterified at the sn-2 position) is given in Figure 2. The incorporation of C18:1n-9c at all given temperatures increased

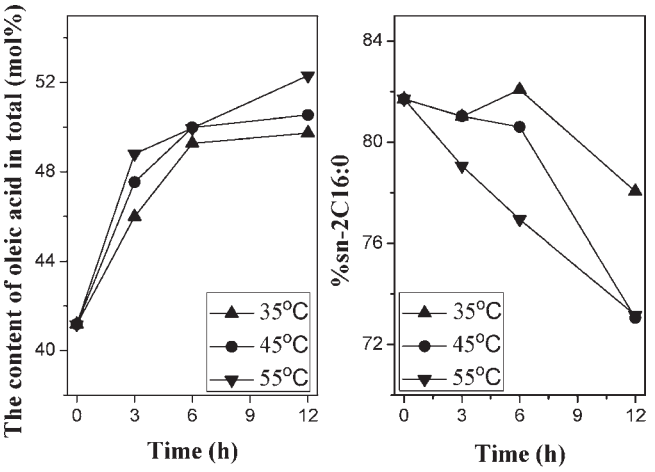

Figure 2. Effect of temperature on the incorporation of C18:1n-9c and on %sn-2C16:0. The reaction mixture contained a substrate molar ratio of 1:4 (34L-leaf lard was kept constant at 1.8 g) and 6% (total weight of substrates) of Lipozyme RM IM. The reaction mixture was incubated at 35, 45, and 55 °C for 12 h in an orbital shaking air bath at 200 rpm.

sharply in the initial 3−6 h of reaction time. The incorporation of C18:1n-9c reached a maximum (52.32%) at 55 °C at the reaction time of 12 h. However, higher temperature significantly decreased the percentage of C16:0 distributed at the sn-2 position. There was an obviously negative correlation between a higher temperature of 55 °C and the percentage of C16:0 located at the sn-2 position ($R = -0.998$, $P < 0.002$). In Figure 2, the percentage of C16:0 esterified at the sn-2 position was still kept at >80% when the temperature was below 45 °C in the first 6 h. Acyl migration, which is unavoidable in lipase-catalyzed reaction, was a serious problem for the production of desired products,[21] especially TAGs enriched with OPO. Considering the incorporation of C18:1n-9c level and the percentage of C16:0 esterified at the sn-2 position in acidolysis products, the reaction temperature of 45 °C was selected for the enzymatic enrichment of OPO in 34L-leaf lard. The migration of C16:0 from the sn-2 into the sn-1,3 positions could be reduced to a very low level at a reaction temperature of 45 °C within 6 h.

Assessment of the Quality of TAGs Enriched with OPO. OPO is considered to be a critical intermediate of human milk fat substitutes or ingredient of infant formulas. It is necessary to evaluate the production of TAGs with a high content of OPO for further use. Until now, HPLC and GC analyses were used to determine the quality of TAGs with high contents of OPO. However, highly demanding technology is required for HPLC analysis of oil and fats,[8,11] and GC analysis is universally used in the oil and fat fields. The deficiency was that only the incorporation of C18:1n-9c into TAGs was investigated, whereas the fatty acid distribution in the chemical structure of OPO product was not considered. Therefore, it is very critical to establish a simple and efficient method to assess the quality of the TAG products enriched with OPO.

In this study, an evaluation model containing these two indices was established by a deducting score principle.[2] Samples such as some published structured lipids, leaf lard, 34L-leaf lard, enzymatic products at different reaction times, and other vegetable oils were used to evaluate the precision of model, and the results are shown in Table 2. It could be seen from sample-1, sample-2, sample-3, and sample-4 that the content of C18:1n-9c was relatively

Table 2. Results of Assessing the Quality of TAGs Enriched with OPO[a]

sample	index	A	B	C	D	E	G_i	G
sample-1[6]	%sn-2C16:0	100	43.85	0.56	1.00	28.08	21.93	51.78
	C18:1n-9c	66.67	39.80	0.40	1.00	20.15	29.85	
sample-2[6]	%sn-2C16:0	100	50.53	0.49	1.00	24.74	25.27	62.32
	C18:1n-9c	66.67	49.40	0.26	1.00	12.95	37.05	
sample-3[7]	%sn-2C16:0	100	58.35	0.42	1.00	20.83	29.18	64.50
	C18:1n-9c	66.67	47.10	0.29	1.00	14.68	35.32	
sample-4[8]	%sn-2C16:0	100	58.53	0.41	1.00	20.74	29.27	62.94
	C18:1n-9c	66.67	44.90	0.33	1.00	16.33	33.67	
leaf lard	%sn-2C16:0	100	86.53	0.13	1.00	6.74	43.27	65.37
	C18:1n-9c	66.67	29.47	0.56	1.00	27.90	22.10	
34L-leaf lard	%sn-2C16:0	100	81.71	0.18	1.00	9.15	40.86	71.74
	C18:1n-9c	66.67	41.18	0.38	1.00	19.12	30.88	
sample-3 h	%sn-2C16:0	100	80.90	0.19	1.00	9.55	40.45	76.22
	C18:1n-9c	66.67	47.70	0.28	1.00	14.23	35.77	
sample-6 h	%sn-2C16:0	100	80.52	0.19	1.00	9.74	40.26	77.73
	C18:1n-9c	66.67	49.96	0.25	1.00	12.53	37.47	
sample-12 h	%sn-2C16:0	100	72.77	0.27	1.00	13.62	36.39	74.24
	C18:1n-9c	66.67	50.47	0.24	1.00	12.15	37.85	
corn oil	%sn-2C16:0	100	7.44	0.93	1.00	46.28	3.72	26.52
	C18:1n-9c	66.67	30.40	0.54	1.00	27.20	22.80	
soybean oil	%sn-2C16:0	100	5.80	0.94	1.00	47.10	2.90	26.37
	C18:1n-9c	66.67	31.30	0.53	1.00	26.53	23.47	

[a] %sn-2C16:0 represents the percentage of C16:0 located at the sn-2 position; A and B represent theoretical and experimental values of the two indices (the percentage of C16:0 esterified at the sn-2 position, the content of C18:1n-9c of total fatty acids), respectively; C represents the floating rate; D represents the weight of each index; E represents the deducting score; sample-3 h, sample-6 h, and sample-12 h represent the results obtained at optimal reaction conditions (substrate molar ratio of 1:4 (34L-leaf lard/camellia oil fatty acids), 6% (total weight of substrates) of Lipozyme RM IM at 45 °C in an orbital shaking air bath at 200 rpm); G_i represents score for index I (%sn-2C16:0) and index II (C18:1n-9c), respectively; G represents total score for different sample.

higher, but that of C16:0 esterified at the sn-2 position was rather low, so the total score was low. Corn oil and soybean oil had the lowest scores for $G_{\%sn\text{-}2C16:0}$ and $G_{C18:1n\text{-}9c}$, which indicated that these two samples contained a small quantity of OPO, showing good agreement with the literature.[22,23] Compared with the total score of leaf lard, 34L-leaf lard showed a higher score, and it was demonstrated that the content of OPO in 34L-leaf lard was enriched after the first stage of concentration by dry fractionation at 34 °C. The scores of enzymatic products produced at different times were analyzed, the highest total score ($G = 77.73$) being obtained when the enzymatic product was produced at 6 h, and it decreased in

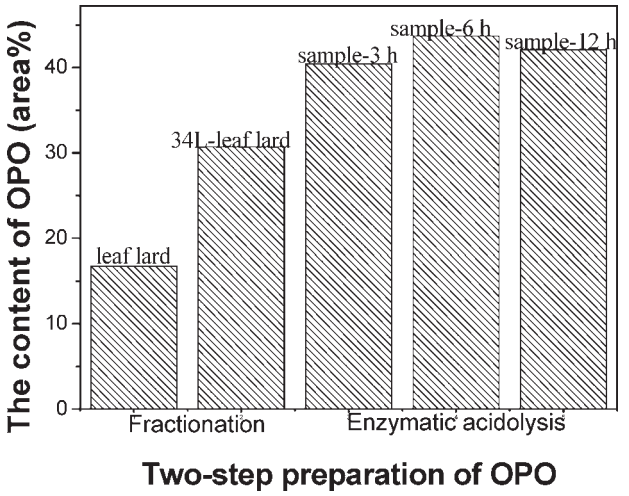

Figure 3. HPLC results of the content of OPO in the first step (fractionation) and in the second step (enzymatic acidolysis).

contrast to the product at 12 h of reaction. This indicated that the OPO was further effectively concentrated after enzymatic process. Figure 3 shows the content of OPO in the initial material (leaf lard) and different products (34L-leaf lard, enzymatic sample-3 h, enzymatic sample-6 h, and enzymatic sample-12 h) by HPLC-ELSD analysis, indicating that the OPO was concentrated effectively after two steps and the OPO content showed a slight decrease when the enzymatic process proceeded to 12 h. This is because more stearoyl-dioleoylglycerol (SOO) and triolein (OOO) were formed with prolonged acidolysis time (data not shown). Similar trends, which were given by HPLC analysis and assessment model, showed that the established model is reasonable.

On the basis of the above results, OPO could be effectively prepared by dry fractionation and a following enzymatic process, and the content of OPO in the final product was improved to 43.72% from the initial content of 16.77%. More importantly, an assessment model was precisely established for evaluating the quality of TAG products with a high content of OPO. The model can be used easily, and only GC data are required. This model could be an efficient way to evaluate the quality of TAG products after different modified processes because it reflected not only the change of total fatty acids but also the distribution of fatty acids.

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