Effect of Palm Stearin Oil (PSO), Physical Fractionation Oil of Palm Stearin Oil (PPP) and Structured Lipids of Rich 1,3-dioleoyl-2-palmitoylglycerol (OPO) on the Stability in Different Emulsion Systems

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Abstract: This manuscript described the preparation of triglycerides with palmitic and ethyl oleate chains, and the stability of emulsions prepared from those triglycerides. Results showed that ratios of total saturated fatty acids (ΣSFA) of palm stearin oil (PSO), physical fractionation oil of palm stearin oil (PPP) and structured lipids of rich 1,3-dioleoyl-2-palmitoylglycerol (OPO) were 72.5%, 95.4% and 33.2% respectively. Rich 1,3-dioleoyl-2 palmitoylglycerol-emulsion (OPO-E) showed a better emulsion stability than that of palm stearin oil (PSO) and physical fractionation oil of palm stearin oil (PPP). The emulsion stability of OPO-E with 10% structured lipids of rich 1,3-dioleoyl-2-palmitoylglycerol (OPO) showed the highest value compared with 5% and 20% OPO. The value of emulsion stability (ES) was 85.5, the values of volume-surface mean diameter ($d_{32}$) and weight mean diameter ($d_{43}$) were 0.09-0.79 μm and 1.1-34.1 μm, respectively. The experimental data had significant ($p < 0.05$) difference with other emulsions. The value of zeta potential ranged from −34.8 to −53.1 mV, indicating that the emulsion stability of 10% OPO was the most stable in all experiment samples.

Key words: palm stearin, physical fractionation oil, 1,3-dioleoyl-2-palmitoyl glycerol, emulsion stability

1 Introduction

According to the different melting point, palm oil can be divided into palm stearin (44-56°C), intermediate component (26-43°C) and palmitin (24°C). After being fractionated by acetone and other organic solvents, palm stearin can be obtained with high-content tripalmitin (PPP) of triglyceride. Tripalmitin and oleic acid (methyl oleate, methyl oleate) can be used to synthesize 1,3-dioleoyl-2-palmitoylglycerol (OPO) structured lipid by enzymatic acidolysis reaction¹ ². OPO could be used as human milk fat substitute (HMFS). And that human milk was a natural emulsion system³.

Human milk fat (HMF) was the richest energy source for infants, supplying about 50–60% of dietary calories. Approximately 98% of the HMF was in the form of triacylglycerol (TAG) with a unique fatty acid tribution. Dietary TAGs are hydrolyzed into 2-monocacylglycerol (2-MAG) and free fatty acids by pancreatic lipase in the intestine. The resulting fatty acids and 2-MAG are absorbed via the intestinal lumen. Saturated fatty acids are better absorbed in the form of MAG than free fatty acids, because 2-MAG is well absorbed by easily forming micelle with bile acids and not forming insoluble soaps with minerals such as calcium and magnesium. Therefore, when palmitic acid was esterified at sn-2 position in the human milk or infant formula, the absorption of palmitic acid and minerals could be improved⁴. Palmitic acid was the major saturated fatty acid, representing about 20–25% of the total fatty acids. It was located at more than 60% sn-2 position of TAG, whereas monounsaturated fatty acid (i.e. oleic acid) was mainly es terified at sn-1,3 positions⁵. HMFS, a lipid resembling TAG of HMF, can be synthesized by an interesterification using a sn-1,3-specific lipase. HMFS containing 69.2% palmitic acid at sn-2 was produced from the blend of tripalmitin, hazelnut oil free fatty acids and stearic acid in hexane cata-
alyzed by Lipozyme RM IM. HMFS (71.1% palmitic acid at sn-2) was prepared from lard and soybean oil fatty acids in solvent-free system in a packed-bed reactor with Lipozyme RM IM\(^\text{[8]}\). To date, a few methods have been developed for monitoring the stability of emulsions, such as optical analysis with microscopy, spectroscopy, turbidity analysis, and particle size analysis\(^\text{[5]}\). But in China, few studies have focused on it. Most domestic need for related products has to rely on imports and the cost is still very high. At present, there have been many researches on the preparation methods of OPO structural esters. However, few studies have investigated the stability of OPO structural esters in emulsion systems, as well as the emulsion stability of different saturated triglycerides. Therefore, the preparation of different content OPO structural esters with different TAG saturations to study the stability of the emulsion system will play an important role in promoting the development of the oil industry.

This study used palm stearin oil and ethyl oleate to carry out ester exchange reaction under the catalysis of lipase TLIM to synthesize OPO. The composition of OPO fatty acid and the structure of triglyceride was explored. Furthermore, the stability of emulsion of palm stearin oil, physical fractionation oil and different saturated fraction of palm was investigated by dynamic analysis, microscopic observation and the analysis of particle size.

2 Experimental Procedures

2.1 Materials and reagents

Market palm stearin oil, Lipozyme TLIM were supplied by Novozyme (Bagsvaerd, Denmark), Lecithin (phosphatidylcholine C 30%), bis[2-hydroxyethyl]amino-tris-[hydroxymethyl]methane (bis-tris), Diaion_HP-20, were purchased from Sigma–Aldrich (St. Louis, MO, USA). All the chemicals and solvents were HPLC-grade and were obtained from Fisher Scientific (Norcross, GA, USA).

2.2 Instruments and equipments

ME104E electronic balance (Mettler Toledo), Constant temperature water bath oscillator, Ultraviolet spectrophotometer, liquid phase (ELSD, Diane, USA), gas phase (Agilent 6890, USA), Mastersizer 3000 Ultra-high-speed intelligent particle size analyzer and Zetasizer Nano HZ New type of dynamic light scattering instrument system (all the instruments are the products of the British Malvern Instruments Ltd).

2.3 Methods

2.3.1 Preparation of PPP rich fraction from palm stearin

PPP was proposed by laboratory method. Palm stearin was mixed with acetone (1:9, w/v), and placed in a temperature controlled chamber at 28°C for 24 hours. Solid fraction was isolated by decanting liquid fraction, and after the evaporation of acetone in solid fraction, a PPP rich fraction was obtained.

2.3.2 Lipid component analysis

OPO and PPP content in the esterification products were analyzed by Agilent 1200 series equipped with an ELSD (SEDEX 80, Alfortville, France) equipment with a column of Discovery Zorbax SB C18 column (250 mm * 4.6 mm *5 μm, Supelco, Bellefonte, USA). The separation of TAG was carried out using a binary solvent gradient program of acetonitrile and dichloromethane. The mobile-phase flow rate was 1 mL/min. The ELSD was held at 55°C and air was used as a nebulizing gas at a pressure of 2.2 bar. TAG samples were dissolved in dichloromethane (1 mg/mL). And 5 mL of samples was the injected. The modified gradientis started at 70% A, decreased to 50% A in 5 min and kept for 25 min, then further decreased to 30% A in 35 min, and finally returned to 70% A in 40 min\(^\text{[3]}\).

First, sample weighed 30 mg and was added with 1.5 mL 0.5 mol/L sodium methoxide. After fully mixed, the reaction lasted for 3 min in 90-degree Celsius. Then, after cooled, the mixture was added with 2 mL BF\(_3\) methanol solution of 14% volume fraction and lasted for 2 min under the condition of 90°C. Next, the solution was cooled and added with 1 mL saturated sodium oxide and isooctane. Fatty acid methyl ester extracted from solution was used in gas phase analysis after dried by anhydrous sodium sulfate. Hewlett-Packard 6890 gas chromatography (GC) equipped with a flame-ionization detector (Agilent Technologies, Little Falls, DE) and chromatographic column was quartz capillary column (SP-2560, 30 m * 0.25 mm, 0.2 μm film thickness, Supelco, Bellefonte, PA) was used to analyze fatty acid composition. GC analysis condition: column temperature was retained at 100°C for 5 min and was improved by heat procedure to be enhanced to 220°C at a rate of a 4°C per minute. When the temperature reaches at 220°C, maintained for 20 min. N\(_2\) was the gas used for transportation. The flow rate of the total gas was 50 mL per minute. The inlet and the detector temperature are 250°C and 260°C respectively\(^\text{[3]}\). The determination method of sn-2 fatty acid content by Schmid\(^\text{[3]}\) with some modifications. Briefly, 2 mL of TriseHCl buffer (1 mol/L, pH 8.0), 0.2 mL calcium chloride (220 g/L), 0.5 mL bile salts (1 mg/mL), 50 mg pancreatic lipase and TAG sample mixture was incubated in a water bath at 40°C for 5 min with vigorous shaking. Then, 1 mL HCl (6 mol/L) and 2 mL of diethyl ether were added. After centrifuge, there were three layers. The first layer which has the diethyl ether was separated and dried with anhydrous sodium sulfate and then evaporated by nitrogen to 500 mL. The hydrolytic products were separated on silica gel GF 254 TLC plates (20 cm×20 cm) and developed with n-hexane/diethyl ether/acetic acid 90:10:1 (v/v/v). The TLC Plates were sprayed with 2,7-di-chlorofluorescein in ethanol (0.2 g/100 mL) and exposed to...
UV light to identify the sn-2 monoglycerides bands. The sn-2 monoglycerides band was then scrapped off carefully and extracted by methylation. Then the fatty acid composition of the sn-2 monoglycerides was analyzed.

2.3.3 Preparation of emulsion

Oil-in-water emulsion (100 g) was prepared with 5, 10, 20% (wt.) OPO in a 20 mM bis-tris buffer solution (pH 7). Lecithin was used as an emulsifier at a concentration of 2.5 wt. % of the OPO-E. The mixture was kept in warm water until pre-homogenized by a Silverson mixer (Model L4RT, Silverson Machines, UK) at a speed of 5000 g for 2 min, and the premix was then passed through a microfluidizer (M-110Y, Microfluidics, MA, USA) with two passes at 5000 psi. The prepared emulsion was placed in the 50 mL vial with caps and stored in room temperature at 20 °C for the emulsion stability study. PSO-E and PPP-E were prepared by the same method, and the oil-water ratio was 10:90 (v/v).

2.3.4 The analysis of dynamic emulsion stability (ES)

The Analysis of dynamic emulsion stability was based on reforming the assay method\(^\text{11}\). Prepared emulsion was centrifuged for 1 hr in the centrifuge and was sampled once per 15 min. 20 μL emulsion samples were diluted with 5 mL buffer solution. The mixed solution was determined by ultraviolet spectrophotometer at a 540 nm wavelength to measure absorbance value. The result was expressed by the velocity formula. The smaller the slope, the greater the reciprocal of the slope calculated, which was calculated to measure absorbance value. The result was expressed by ultraviolet spectrophotometer at a 540 nm wavelength to measure absorbance value. The result was expressed by the velocity formula. The smaller the slope, the greater the reciprocal of the slope calculated, which was calculated by the velocity formula. The smaller the slope, the greater the reciprocal and the greater the stability became.

\[ \ln \left( \frac{E_A}{E_{A_0}} \right) = K_d \times t \quad (1) \]

\[ ES = - \frac{1}{K_d} \quad (2) \]

Where \(E_{A_0}\) in Eq. (1) is the absorbance at the initial time (\(t = 0\)). ES is described by the slope of the \(\ln(E_A/E_{A_0})\) versus \(t\) plot. Since the smaller the \(K_d\), the greater the stability is, the larger ES value means greater stability according to Eq. (2). Duplicate analyses were performed.

2.3.5 The analysis of particle size

Average particle sizes of emulsion were determined by Mastersizer 3000 Ultra High-Speed Intelligent Particle Size Analyzer (British Malvern Instruments Ltd). Results were expressed in \(d_{43}\) and \(d_{32}\).

\[ d_{43} (\mu m) = \sum d_i^3/\sum d_i^2 \times n_i \quad (\text{ni: The number of particles, di: diameter}) \]

\[ d_{32} (\mu m) = \sum d_i^2/\sum d_i^2 \times n_i \quad (\text{ni: The number of particles, di: diameter}) \]

2.3.6 Rheological measurements

Rheological measurements involving extended rotational and oscillatory tests were performed with a Physical MCR 301 rheometer equipped with air bearing and parallel plate measuring geometry with 1 mm gap, and precise temperature controller. Rotational tests in a controlled shear stress (CSS) mode were performed to reveal the rheological behavior of the studied materials in the shear flow conditions.

2.3.7 The Observation of electric potential

The Zeta potential on the surface of the emulsion was measured by using the new top dynamic light scattering system. The emulsion was sampled and was diluted to 1% concentration by adding a buffer.

2.3.8 Statistical analysis

The difference in significant data was used F test method and the Duncan test method in SPSS 19.0 software to carry on the multiple comparisons (\(p < 0.05\)).

3 Results and Discussion

3.1 Triglyceride structure and fatty acid composition analysis

In Fig. 1, the content of OPO from A to G was 11.5%, 3.5%, 25.3%, 29.1%, 26.7%, 26.3%, 21.2% and 21.2%, respectively. Theoretical values of sn-2 Palm Acid are 93.1%, 94.9%, 61.0%, 53.9%, 56.1%, 55.1% and 36.2% respectively. Although the content of POP in sample B (64.2%) was significantly higher than sample A (33.3%), both theoretical value content of sn-2 position palm acid was similar (93.1% and 94.9%). Thus the sample A should be selected as crude oil to react with ethyl oleate under 55°C (1:3, mol/mol). The reactant was mixed with 10% lipase lipzyme TL IM to obtain 1,3-dioleoyl-2-palmitoyl glycerol. The reaction lasted for 6 hours. sn-2 position palm acid (C16:0) content of 1,3-dioleoyl-2-palmitoyl glycerol reached 54.5% and sn-1,3 oleic acid (C18:1) content of it reached 73.8% (Table 1). The final product was the human milk substitutes whose 1,3-dioleoyl-2-palmitoyl glycerol reached 29.1%. Our results were similar to those of Lee et al.\(^\text{12}\) They were using acetone as the solvent to fractionate palm stearin and OPO (31.4%) was produced by the reaction between physical fractionation oil and ethyl oleate.

3.2 Analysis of emulsion stability (ES)

Data presented in Fig. 2 was calculated by the following formulas, such as \(\ln (E_A/E_{A_0}) = K_d \times t\) and \(ES = -1/K_d\).
The value of the ES in the Fig. 2 was PSO-E (116.2), PPP-E (833.3), 5% OPO-E (87.0), 10% OPO-E (85.5), 20% OPO-E (86.9) respectively. The ES value of the PPP emulsion was significantly different with a significant difference in the value of PSO, 5% OPO, 10% OPO and 20% OPO emulsions (p < 0.05). There was no significant difference (p > 0.05) among the emulsion of 5% OPO, 10% OPO and 20% OPO. PPP emulsion has high saturated fatty acid (SFA) content, and also the highest ES value. These results indicated higher SFA content would be followed with high ES value. But the results of this experiment were contrary to those of Zhang et al. Wherein ES generally measures the amount of oil and/or cream separated from an emulsion over a short period, calculated as the turbidity of emulsion, dependent on the oil concentration, gravitational field, and temperature. High ES value of PPP with higher saturation may be the cause of high turbidity.

3.3 Emulsion particles size change
The average diameter (d_{32}) refers to the ratio between all the dispersed phase droplets in the emulsion and the total surface area. Generally, the smaller emulsion size, the greater surface area became. Besides, higher degree of dispersion was also observed in the same oil volume. The emulsion was prepared by micro fluidic and was stored in the room temperature (20°C), to observe the change of particles’ size for 24 days (Tables 2 and 3). On 0 (zero) day, the d_{32} of the emulsion was ranged between 0.07-0.35 μm.
except the PPP’s (14.1 μm). The PSO emulsion of d32 (9.07 μm) on the sixth day increased significantly. The d32 of 5% OPO and 20% OPO on the 24th day increased quickly. However, the d32 of 10% OPO on the 24th day was still 0.79 μm. It has been reported that greater emulsion stability is obtained with lower d32 and d43 values, suggesting that 10% OPO emulsion demonstrated a higher emulsion stability than other emulsion, and was not easy to form flocculation phenomenon. In addition, the d43 and d32 values in the 20% OPO emulsions of oil mass high and PPP emulsions of saturation content high steadily increased with same emulsifier content.

3.4 Microscopic observation

Furthermore, to have a full view of the emulsion microstructure, we used optical microscopy to confirm the emulsion dispersed state (Figs. 3 and 4). As shown in Fig. 3, 5% OPO, 10% OPO, and 20% OPO emulsions all produced some phenomenon of the partial aggregation on the 10th day.
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Fig. 3 Microscopy images of PSO-E, PPP-E, 5% OPO-E, 10% OPO-E, 20% OPO-E emulsions at 1 day (×100).

Fig. 4 Microscopy images of PSO-E, PPP-E, 5% OPO-E, 10% OPO-E, 20% OPO-E emulsions at 10 days (×100). Scale plate of 100 μm.

Fig. 5 (Color online) Viscosity curves of emulsions with PSO-E, PPP-E, 5% OPO-E, 10% OPO-E, 20% OPO-E, (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

20% OPO emulsion produced the large drop size at the same time. The presence of individual droplets with variable sizes not only may influence the average particle size but also could be a first symptom of emulsion destabilization.

Five of the emulsions presented in Fig. 5, i.e. PSO-E and 20% OPO-E possessed extended plateau region at lower shear rates, while for the others it was less pronounced. Such rheological behaviors are well described by a Cross equation: \( \eta = \eta^\infty + (\eta_0 - \eta^\infty)/(1 + \alpha \gamma^p) \), which gives the values of zero shear viscosity (\( \eta_0 \)) at shear rates approaching zero and limiting viscosity (\( \eta^\infty \)) at infinitely high shear rates; \( \gamma \) — shear rate, \( \alpha \) — relaxation time and \( p \) are experimentally derived model parameters. Curve fitting parameters according to the cross and power law equations. It could be seen from the combination of Figs. 3 and 4, high viscosity will form flocculation (PSO-E), high oil content will form large droplets (20% OPO-E) were formed storage time.

As the shear stress, the stability of the emulsion was
strong related to the oil and saturated ratio. In our case, despite the same amount of emulsifier was added, the emulsions with different oil content and saturation showed different stability, indicating flow behavior of the emulsions with the same emulsifier content is influenced by both the saturated content and oil content. High saturated content in the case of PPP-E in comparison to the other emulsions with the same emulsifier content, leads to formation of emulsion with higher dispersity index and average droplet size.

3.5 Potential observation

Zeta potential was widely used to reflect the strength of mutual repulsion or attraction between particles. Besides, the value of Zeta potential was closely related to the stability of the emulsion stability. As had been reported, zeta potential of the stable emulsion was generally higher than 30 mV. From Fig. 6, it can be seen that zeta potential of 5% OPO, 10% OPO and 20% OPO emulsions was higher than the PSO and PPP emulsion systems. It revealed that the system was more stable. When OPO was dissolved or dispersed in a solvent, it could resist aggregation. Because the zeta potential (negative value) of PSO and the PPP emulsion was relatively low, they tend to condense into flocculation. Namely, attraction exceeded repulsive forces. Then, the dispersion was destroyed and flocculation or aggregation occurred.

4 CONCLUSIONS

Taken together, the present study investigated the stability of different emulsion systems including palm stearin oil (PSO), physical fractionation oil (PPP) and structured lipids (OPO). The main findings are as follows: (1) under the condition of adding 10% (w%) lipase TL IM, the synthetic reaction between palm stearin and oleic ethyl ester (1:3, mol/mol) at 55°C lasted for 6 h. The synthetic structured lipids were the most consistent with human milk substitutes. The total saturated fatty acid of PSO, PPP and OPO were respectively 72.5%, 95.4% and 33.2%. It was closely related to emulsion stability. (2) Based on the results of particle size determination, microscopic observation, electric potential value and other indexes, 10% OPO was the most stable emulsion system. It has been of great urgency to focus on the artificial infant formula food with OPO simulating human milk system and to study emulsion stability worldwide. The present study can provide useful information to enhance the absorption of essential fatty acids and minerals for infant with lower prices.

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