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Immobilised *Aspergillus niger* lipase synthesises *sn*-1,3-dioleoyl-2-palmitoylglycerol

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Abstract: *sn*-1,3-Dioleoyl-2-palmitoylglycerol (OPO) is a structural triglyceride with a specific distribution of fatty acids, which is an important component of breast milk fat and which can better promote the absorption of minerals and energy in infants. In this study, a lab-made immobilised *Aspergillus niger* lipase (IM-ANL), which is more economical than a commercial lipase, was used for hydrolysis to produce free fatty acids (FFA) and acidolysis interesterification to produce OPO. The enzyme was used for multiple purposes, reducing the cost of enzymatic production. The optimum conditions for hydrolysis were determined by experiments: the amount of IM-ANL enzyme dosage was 3%, the reaction temperature was 45 °C, and the reaction time was 48 h. The optimum conditions for acidolysis interesterification were as follows: the amount of IM-ANL enzyme dosage was 4%, the molar ratio of tripalmitin to oleic acid was 1 : 8, the reaction temperature was 55 °C, and the reaction time was 3 h. In this study, economical palm stearin and high oleic acid sunflower seed oil were selected as reaction raw materials, and the reaction temperature was moderate, the safety risk was low, the energy consumption was reduced, the process was more economical, and the economic value of high oleic acid vegetable oil was improved, which was conducive to the further promotion of OPO production.

Keywords: lipase immobilised enzyme; hydrolysis; acidolysis; interesterification; *sn*-1,3-dioleoyl-2-palmitoylglycerol

Breast milk contains a large number of essential nutrients for infant growth and development. Lipid is one of the important nutrients in breast milk, and its main function is as an energy source for infants. Fat contained in breast milk can provide about 50% of the energy for infants with exclusive breastfeeding (Fenton et al. 2021), participates in the rapid growth of multiple organs of infants, and helps to maintain the normal gastrointestinal function of infants (Ramiro-Cortijo et al. 2020). It promotes the nervous system development and immune system development (Dimitroglou et al. 2022).

About 98% of the lipids in breast milk are in the form of triglycerides (TAG) (Innis 2011). The structure of triglycerides will affect the absorption of fat, and each position is named *sn*-1, *sn*-2 and *sn*-3 according to the ordinal number of carbon atoms of glycerol, about 70% of the saturated fatty acids in breast milk triglycerides are distributed at the *sn*-2 position, and the unsaturated fatty acids are mainly located at the *sn*-1,3 position (Wei et al. 2021). Studies have shown that 1,3-dioleoyl-2-palmitate triglyceride (OPO) is an important component of breast milk fat, and the content of OPO in breast milk is 16% to 29% (Kallio et al. 2017). Palmitic acid bound to *sn*-1,3 in vegetable oil and infant formula is not easily absorbed. It is easy to form insoluble calcium soap that is discharged from the body with faeces, resulting in the loss of energy and calcium (Wei et al. 2019). Because of its unique fatty acid structure composi-

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tion, oleic acid (OA) is esterified at the *sn*-1,3 positions of OPO, where it is not easy to form calcium soap when participating in digestion and when it causes constipation. OPO can promote the absorption of calcium and other minerals in infants, promote the formation of soft stools, relieve infants' crying (Miles et al. 2017), and make infants better absorb minerals and energy (Ghide et al. 2021). In 2008, Announcement No.13 of the Ministry of Health of the People's Republic of China approved OPO as a nutritional fortification agent that can be used in infant formula milk powder, and specified its production technical indicators and strengthening amount. In 2015, the Health and Family Planning Commission of the People's Republic of China issued the National Food Safety Standard GB30604-2015 (National standard for food safety 2015) for OPO as a food nutrition fortifier, which stipulates that OPO is made from edible plant oil and oleic acid (derived from edible plant oil) as raw materials, and is prepared by lipase catalysed esterification in the food industry. Therefore, it is of great practical significance to study the enzymatic synthesis of OPO structural lipids.

At present, most literature sources report the use of commercial immobilised enzymes, such as Lipozyme RM IM, Lipozyme TL IM, Novozym 435, NS40086 etc., which have high cost and are mainly used only for the acidolysis of OPO production (Martins et al. 2014). In addition, the raw material FFA used in the production of OPO also requires the hydrolysis of other lipases. The use of immobilised *Aspergillus niger* lipase has not been seen. *Aspergillus niger* is a typical filamentous fungus, which belongs to *Deuteromycotina*, *Hyphomycetes*, *Hyphomycetales*, and *Moniliaceae*. It is a common species in the genus *Aspergillus*. It is recognised as GRAS (Generally Recognised As Safe) by USFDA (U.S. Food and Drug Administration) and has good biological safety (Chakraborty et al. 2023). *Aspergillus niger* is widely distributed in food, plant-based products and soils worldwide. Humans have a long history of using *Aspergillus niger*. In ancient times, people used *Aspergillus niger* to make wine, sauce, vinegar and so on (Li et al. 2020). Lipase produced by *Aspergillus niger* is an important industrial enzyme, which is widely used in food, chemical and pharmaceutical industries.

The immobilised *Aspergillus niger* lipase (IM-ANL) can be used to produce free fatty acid (FFA) in the pre-hydrolysis reaction and OPO in the post-acidolysis reaction. The multipurpose enzyme implies the better economic value. In this study, the OPO structure lipid was synthesised by using palm stearin (fractionated at 52 °C) and high-oleic sunflower oil (HOSO) as substrate and IM-ANL as catalyst. The influence of temperature, molar ratio of the substrate, time and enzyme dosage on the change of lipid content of OPO during the reaction was discussed, and the reaction conditions were optimised.

MATERIAL AND METHODS

Experimental material

Raw materials and reagents. Palm stearin [melting point: 52 °C, primarily consisted of tripalmitin (PPP, 40–60% w/w), 1-oleoyl-2,3-dipalmitoyl-*sn*-glycerol (OPP, 25–40% w/w) and OPO (5–7% w/w), with an acid value below 1.0 mg $KOH \cdot g^{-1}$] was purchased from Kerry Oil & Grains (Tianjin) Co. (China), Ltd; HOSO was purchased from Liaoning Shengmai Industrial Co., Ltd (China); Qingdao Vland Biotech Co., Ltd. (China) supplied *Aspergillus niger* lipase (ANL); the ANL immobilization (ANL was immobilised on resin via adsorption crosslinking, yielding 12 000–15 000 $U \cdot g^{-1}$ activity). The immobilised enzyme that retained >85% activity after 10 cycles and exhibited enhanced pH/thermal stability (pH 4–9, 60 °C) was made by Synthetic Biology Engineering Center of Chengdu Skuny BioScience Co., Ltd (China); n-hexane, methanol and tetrahydrofuran were chromatographically pure; ethanol, diethyl ether and sodium hydroxide were analytically pure.

Instruments and equipment. Shimadzu GC-2014C gas chromatograph and high-performance liquid chromatograph, Shimadzu Instrument (Suzhou) Co., Ltd (China); DF-101 thermostatic water bath, Gongyi Yuhua Instrument Co., Ltd (China); JB300-SH digital display constant speed electric mixer, Shanghai Specimens and Model Factory; electronic analytical balance, Shenyang Longteng Electronics Co., Ltd. (China).

Experimental method

Enzymatic hydrolysis to produce free fatty acids. A 1 000 g aliquot of HOSO was subjected to enzymatic treatment at 50 °C. IM-ANL was added to the oil at concentrations of 0.5, 1, 3, 5, and 7% (w/w) and mixed by mechanical stirring at 150 rpm for 20 min. Subsequently, deoxygenated water (1 : 1, w/w to oil) was added to the mixture. The temperature optimisation experiments were initiated at 35 °C and increased by 10 °C increments up to 75 °C, with continuous mechanical stirring at 300 rpm. During the 48-hour hydrolysis reaction, samples were collected at 3-hour intervals for acid value determination, then they were left to stand to remove the water layer, the oil layer was collected and weighed. Fresh deoxygenated water

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of the same weight as collected oil was added and the previous operation was repeated to collect sunflower seed hydrolysed oil. The collected oil was removed by rotary evaporation. Acid value, triglyceride content and fatty acid composition were determined.

Pretreatment of palm stearin. The palm stearin was placed in a constant temperature water bath at 75 °C, until it was completely dissolved into the liquid, and then stirred for 20 min to mix well. The contents of OPP and PPP in palmitate stearate were determined by high-performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD) in 1 mL samples. The GB 5009.168-2016 method quantifies OPP and PPP in oils via HPLC-ELSD using a C18 column with acetonitrile-isopropanol gradient elution (1.0 mL·min^{-1}, 30 °C). Samples are dissolved in n-hexane (10 mg·mL^{-1}), detected by ELSD (45 °C, 3.5 bar), and quantified against certified standards with 0.05% LOD.

Interesterification – enzymatic acidolysis. The pretreated palm stearin (primarily containing PPP and OPP) was mixed with HOSO free fatty acids (HOSO FFA) [predominantly containing OA, OA and linoleic acid (LA) that accounted for 83.95% and 7.41% of the total fatty acids, respectively] at molar ratios of 1 : 2, 1 : 4, 1 : 8, 1 : 10 and 1 : 20, respectively, placed in a constant temperature water bath and mixed by a mechanical stirring at 250 rpm. Reactions were conducted using IM-ANL at 0.5, 2, 4, 6, and 8% (w/w), with the temperature initially set at 45 °C and increased by 5 °C increments up to 65 °C. The reaction time was fixed at 24 h, and samples were collected at nine predetermined time points for analysis, and the final reaction products were collected and sealed with nitrogen.

Molecular distillation separation of products. Due to different boiling points, TAG and FFA are separated from the reaction products by molecular distillation, FFA is obtained from the distillate, TAG is obtained from the residue, and the product OPO exists in the residue. Molecular distillation conditions: injector temperature 70 °C, evaporation surface temperature 165 °C, condenser temperature 65 °C, pressure 3–5 Pa, rotary scraping film speed 1 000 rpm, feed once, return twice, three feed speeds $V1$ = 10 mL·min^{-1}, $V2$ = 5 mL·min^{-1}, $V3$ = 3 mL·min^{-1}.

Acid value measurement and calculation. According to Method I for acid value determination (free fatty acids) in the United States Pharmacopeia (USP 40, Chapter <401> 'Fats and Fixed Oils'), the sample solvent was prepared by mixing ethanol and diethyl ether at 1 : 1 (V/V). An amount of 0.2 g sample was accurately weighed, and 10 mL of sample solvent was added, then 0.2 mL of 1% (W/V) phenolphthalein indicator solution was added, and the mixture was evenly oscillated. An amount of 0.1 mol·L^{-1} of potassium hydroxide standard titration solution was used for titration until the sample solution turned light pink and did not fade after oscillating for 30 s, and the amount of consumed potassium hydroxide titration liquid was recorded.

$$\text{Acid value (KOH mg·g}^{-1}) = 56.11 \times V \times C/W \quad (1)$$

where: W – sample weight (g); V – $V1$ – $V0$ (titration liquid product consumed by the sample minus titration liquid product consumed by the blank) (mL); C – potassium hydroxide standard titration solution concentration (mol·L^{-1}).

The theoretical hydrolysis rate can be derived based on the molar quantity of fatty acids (molecular weight 280–282.5 g·mol^{-1}) bound in triacylglycerols (TAG, molecular weight 884.5 g·mol^{-1}). The amount of the substance TAG in 1 000 mg of oil is calculated as follows:

$$nTAG = 1\,000 \text{ mg} / 884.5 \text{ g·mol}^{-1} = 3.392 \text{ mmol} \quad (2)$$

The acid value (AV) reflects the molar quantity of free fatty acids, which can be converted using the molecular weight of potassium hydroxide (56.1 g·mol^{-1}):

$$\text{nfree fatty acids} = AV / 56.1 \quad (3)$$

Therefore, the degree of hydrolysis (DH) is calculated in the following way:

$$DH = [AV / (56.1 \times 3.392)] \times 100\% \quad (4)$$

Quantification of triacylglycerol (TAG) content by high-performance size-exclusion chromatography (HPSEC). The column was made of stainless steel with a length of 300 mm and an inner diameter of 7.5~7.8 mm. The temperature of the oven was 35 °C and the sample volume was 10 μL. The mobile phase was tetrahydrofuran and the cleaning solution was 10% methanol solution; flow rate 0.8 mL·min^{-1}; pump pressure 7.9 MPa; isocratic elution.

Determination of fatty acid distribution at sn-2 position. According to the methods of ISO 6800:1997, weigh 0.1 g of the sample in a 10 mL centrifuge tube, add 2 mL of 20 mg pancreatic lipase and 1 mol·L^{-1} Tris buffer (pH 8.0), carefully shake well, then add 0.5 mL of sodium cholate solution (2 g·L^{-1}) and 0.2 mL of calcium chloride solution (220 g·L^{-1}), shake

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for 3 min in a water bath at 40 °C. The reaction was terminated, and 1 mL of 6 mol·L^{-1} hydrochloric acid and 1 mL of diethyl ether were added immediately, shaken vigorously, and finally centrifuged at 2 795 × g for 10 min. The upper layer was removed, and after addition of a small amount of anhydrous sodium sulphate, the upper layer was transferred again, and the solution was concentrated to one-third of its original volume under a gentle stream of nitrogen gas. The concentrated samples were separated by thin layer chromatography with n-hexane-diethyl ether-acetic acid (50 : 50 : 1) as the development agent, and the colour was developed with 0.2% 2,7-dichlorofluorescein under ultraviolet light. The isolated *sn*-2 fatty acids were extracted with ether, methyl esterified and analysed by gas chromatography.

Fatty acid content was determined by methyl esters. According to the methods of ISO 12966-2:2017 and ISO 12966-1:2014, take the sample to be tested, add 2 mL of 0.5 mol·L^{-1} sodium hydroxide methanol solution, mix well, boil for 30 min, connect to the condenser, add 1 mL of boron trifluoride methanol solution, continue heating for 3 min, then add 20 mL of sodium chloride solution. After shaking violently for 30 s, 2 mL of isooctane was added, mixed evenly, and then layered. The supernatant was taken, and an appropriate amount of anhydrous sodium sulphate was added to remove the residual water in the solution. Finally, the membrane was filtered and analysed by gas chromatography.

OPO content was detected by gas chromatography. The chromatographic column was DB-1HT, 30 m × 0.25 mm × 0.10 μm. Detector: FID detector (Agilent Technologies, USA), temperature: 380 °C; oven temperature: 300 °C; inlet temperature: 320 °C; column equilibration: 3 min at initial temperature after each run; injection method: shunt; injection volume: 1 μL; sampling speed: 40 ms; carrier gas: nitrogen (purity ≥ 99.999%) at 1.0 mL·min^{-1}; FID gases: H_2 (40 mL·min^{-1}), synthetic air (400 mL·min^{-1}); control mode: line speed; pressure: 159.7 kPa; total flow rate: 54.1 mL·min^{-1}; linear velocity: 34.4 cm·s^{-1}; purge flow rate: 3.0 mL·min^{-1}; split ratio: 50 : 1.

RESULTS AND DISCUSSION

Effect of enzyme amount on hydrolysis reaction

The extent of sunflower seed oil hydrolysis can be characterised by monitoring the DH of the products. The content of free fatty acid (FFA) in the product increases, so the acid value of the product increases (Chen et al. 2023). As shown in Figure 1, this is the result of the reaction in 15 hours during the first hydrolysis, when the amount of the enzyme is low, the contact probability of the reactant and the active site of the enzyme is low, resulting in the reduction of catalytic efficiency and the slow increase of DH. In the same reaction system, the contact surface between the reactant and the active site of the enzyme is limited, and when the amount of enzyme is high, the rate of DH increase cannot be significantly improved, and the catalytic efficiency is not significantly improved. From the perspective of cost saving, when the mass ratio of oil and water in the reactant is 1 : 1, and the reaction is carried out by mechanical stirring at 250 rpm, at 50 °C for 15 h, 3% of the amount of enzymatic hydrolysis is the most appropriate.

Effect of temperature on hydrolysis reaction

As shown in Figure 2, the enzyme dosage was 3% (w/w), the DH of sunflower seed oil was the highest at 45 °C and the lowest at 75 °C during the hydrolysis reaction for 12 h. As the reaction temperature increases, the molecular movement in the system accelerates, and the contact probability between the enzyme and the substrate increases, which is conducive to the hydrolysis reaction. When the temperature is too high, the advanced structure of the enzyme will change or denature, resulting in reduced or even lost enzyme activity (Druteika et al. 2020). After the reaction at 75 °C for 3 h, the DH has not changed after reaching 19.27%. Therefore, it is appropriate to main-

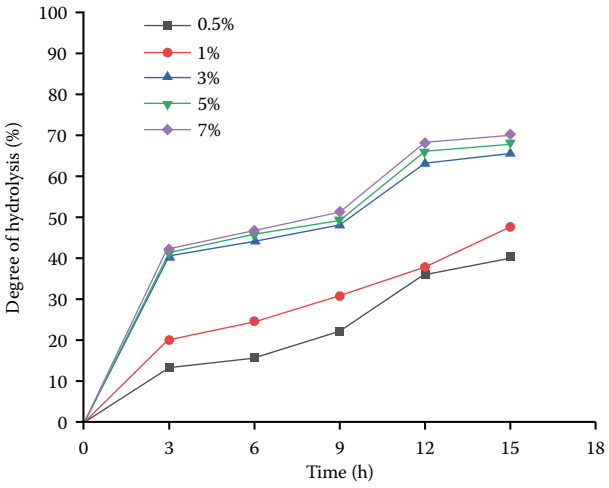

Figure 1. Effect of enzyme amount on degree of hydrolysis change in hydrolysis reaction

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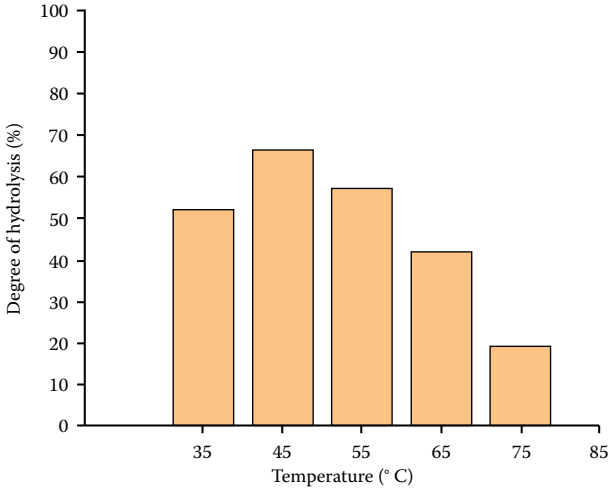

Figure 2. Influence of reaction temperature on degrees of hydrolysis change

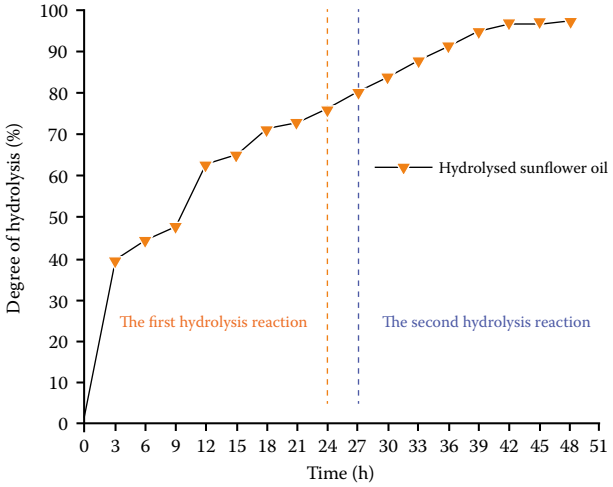

Figure 3. Change of degree of hydrolysis with reaction time

tain the temperature of IM-ANL hydrolysis at 45 °C and the DH can reach 66.38%.

DH and TAG change with reaction time

With the increase of reaction time, the FFA content in the product increased, and the acid value increased, causing a decrease in the pH value of the water in the reaction system (Chen et al. 2023). The FFA generated during hydrolysis partially dissociate, releasing protons (H^+). The accumulation of H^+ directly lowers the pH of the aqueous phase (increased acidity). The optimum pH value of ANL was less than 7, which was acidic (Zhu et al. 2020), but the optimum pH value of IM-ANL immobilised on the resin support was more than 7, which was weakly alkaline (El-Ghonemy et al. 2021). So as shown in Figure 3, at 24 h reaction, the DH is 75.99%, the oil and water are separated, and then re-injected with fresh deoxygenated water of equal oil quality, and the reaction continues for 24 h, the DH is 97.26%. Since the pH of the fresh deoxygenated water is neutral, and the glycerol in the product of the first hydrolysis reaction is separated, so the second hydrolysis reaction can continue. The TAG content of the product was detected by HPSEC. As shown in Figure 4, FFA gradually increased and TAG gradually decreased with the hydrolysis reaction. Since TAG was 1.89% at 48 h, the hydrolysis of residual TAG in the substrate became extremely difficult due to the small probability of contact between the enzyme and the residual TAG, so the reaction was terminated (Fan et al. 2023). The residual TAG was separated by molecular distillation, and the distilled FFA were collected as feedstock for subsequent interesterification acidolysis.

Effect of substrate molar ratio on acidolysis interesterification

The acidolysis reaction was performed using IM-ANL-catalysed HOSO FFA (acid value: 185.11 mg $KOH \cdot g^{-1}$; degree of hydrolysis: 97.26%) and palm stearin as substrates. Gas chromatographic analysis revealed that the palm stearin contained 36.25% OPP and 41.29% PPP, while the methyl-esterified hydrolysate of HOSO contained 83.95% oleic acid (OA). The reactants were added in weight gradients, with the calculated molar ratios

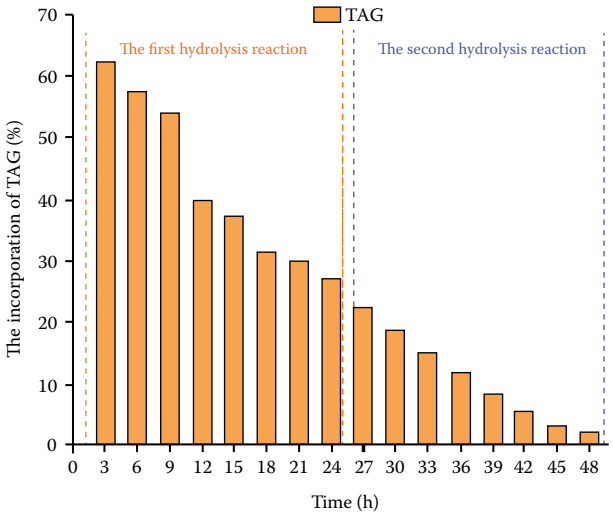

Figure 4. Changes of triglycerides (TAG) with reaction time

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of PPP (considering OPP as half-equivalent PPP) to OA being approximately 1 : 2, 1 : 4, 1 : 8, 1 : 10, and 1 : 20. IM-ANL (4% of the substrate weight) was added, and the reaction was subjected to acidolysis for 12 h at 55 °C and 250 rpm with mechanical agitation. ANL catalyses the acidolysis of palmitoyl groups at *sn*-1,3 positions with oleic acid via a three-step mechanism:

i) formation of a Michaelis complex between the ANL catalytic triad (Ser-His-Asp) and TAG,

ii) nucleophilic attack by Ser to form a palmitoyl-enzyme intermediate, and

iii) interesterification by oleic acid to produce OPO (Peng et al. 2022).

Because of the *sn*-1,3 position selectivity of IM-ANL, OA preferentially reacted with palmitic acid (PA) at *sn*-1,3 position, and it was difficult to react with PA at *sn*-2 position (Hasibuan et al. 2021). Theoretically, when the molar ratio of PPP and OA is 1 : 2, and the molar ratio of OPP and OA is 1 : 1, complete acidolysis can occur, but this reaction is reversible, and the OPO generated can also regenerate PPP or OPP with the replaced PA. Therefore, increasing the proportion of acyl donor (acid) appropriately can make the reaction balance move to the right. It is beneficial to the accumulation of OPO (Li et al. 2023). As shown in Figure 5, the optimal reaction was achieved when the substrate molar ratio was 1 : 8.

Effect of temperature on acidolysis interesterification

Considering that the optimum temperature of thehydrolysis reaction of IM-ANL is 45 °C, but when the temperature is lower than 45 °C in the acidolysis, the mixture of palm stearin and HOSO FFA will appear solidified, so the temperature gradient experiment of acidolysis is selected above 45 °C, and the molar ratio of PPP and OA is about 1 : 8, and the reaction time is 12 hours. The effect of temperature on the OPO content of the product is shown in Figure 6, with the best reaction at 55 °C. The enzymatic catalytic efficiency was relatively low in the 45–50 °C range. When the temperature increased to 60–65 °C, the elevated temperature tended to induce enzyme denaturation. The catalytic efficiency of the enzyme was low at a low temperature. When the temperature is too high, the structure of the enzyme is affected by high temperature, resulting in reduced enzyme activity and decreased reaction efficiency (Kumar et al. 2020).

Effect of IM-ANL enzyme amount on acidolysis interesterification

When the enzyme loading was 0.5–2%, the OPO content remained below the target value. Increasing the enzyme concentration to 4–8% did not significantly enhance the OPO content. When the amount of the enzyme is low, the reactant is less likely to contact the active site of the enzyme, resulting in lower catalytic activity. Since the acidolysis is reversible, it is difficult to increase the OPO content over time as the reaction system tends to equilibrium (Gao et al. 2022). When the amount of the enzyme is too large, although the OPO content can be increased faster, there is not much advantage in terms of the increase range, and the enzyme amount is too large to increase the cost of the

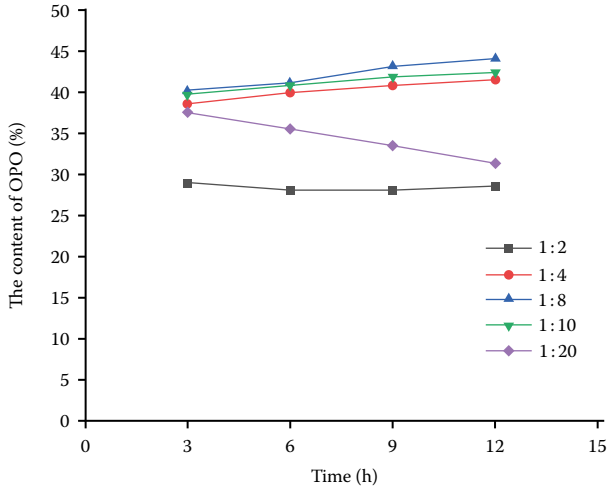

Figure 5. Effect of the PPP-to-OA molar ratio on the variation of OPO content

PPP – tripalmitin; OA – oleic acid; OPO – 1,3-dioleoyl-2-palmitate triglyceride

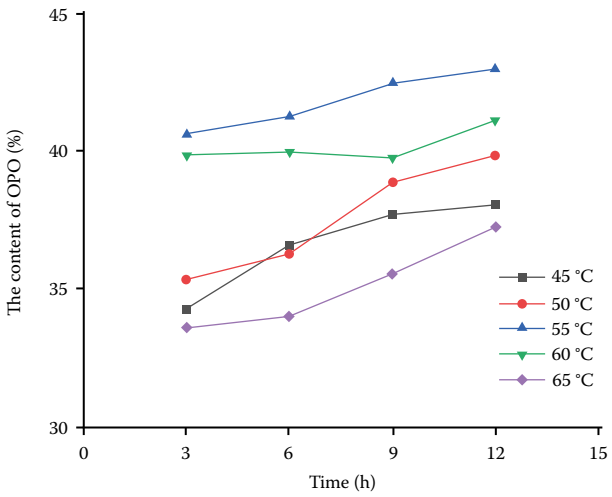

Figure 6. Influence of reaction temperature on the change of 1,3-dioleoyl-2-palmitate triglyceride (OPO) content

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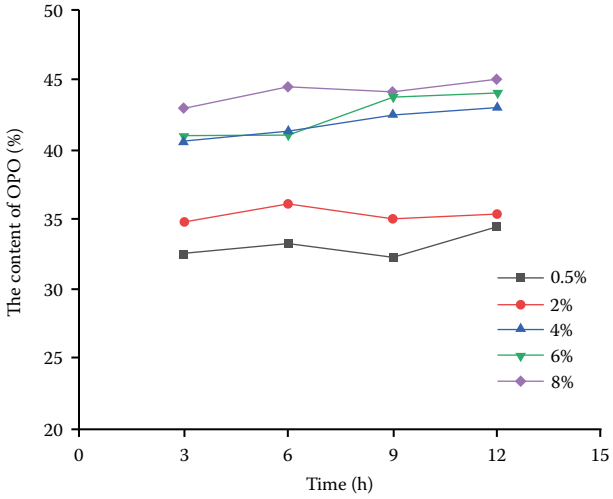

Figure 7. Influence of enzyme amount on acidolysis

OPO – 1,3-dioleoyl-2-palmitate triglyceride

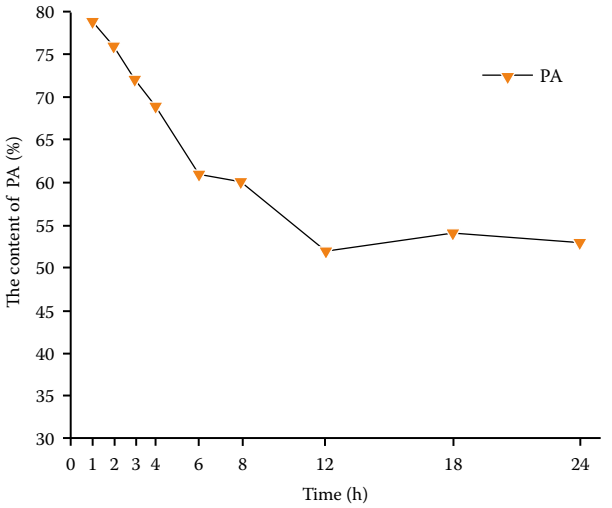

Figure 9. Effect of time on the change of palmitic acid (PA) content at *sn*-2 position

reaction. Therefore, it is important to optimise the amount of the enzyme. When the molar ratio of PPP and OA is about 1 : 8, the reaction is carried out at 55 °C and 250 rpm for 12 h with different enzyme amounts, as shown in Figure 7. Since GB30604-2015 (National standard for food safety 2015) requires OPO content ≥ 40%, considering economic factors, the enzyme amount of 4% is the most reasonable.

Effect of reaction time on acidolysis interesterification

The OPO content increased continuously during the initial 8-hour reaction period, reaching its maximum at 8 h. Prolonging the reaction to 24 h resulted in a slight decrease in OPO content. The acidolysis to produce OPO is a reversible reaction. Therefore, when the palmitic acid (PA) at *sn*-1,3 position of PPP is replaced by OA, the free PA in the reaction system will increase over time, and the free PA will also displace the OA at *sn*-1,3 position of the product OPO, thus slowly reaching an equilibrium (Ghide et al. 2022). At this time, if the reaction continues, the content of OPO will be difficult to increase or even decrease, and the PA at *sn*-2 position is more prone to acyl migration during the reaction, resulting in the decrease of PA content at *sn*-2 position (Cai et al. 2015). Therefore, as shown in Figures 8 and 9, considering the requirement of OPO content ≥ 40% and the content of PA at *sn*-2 position ≥ 52% in GB30604-2015 (National standard for food safety 2015), as well as the process efficiency, the reaction time of 8 h was selected as the most appropriate.

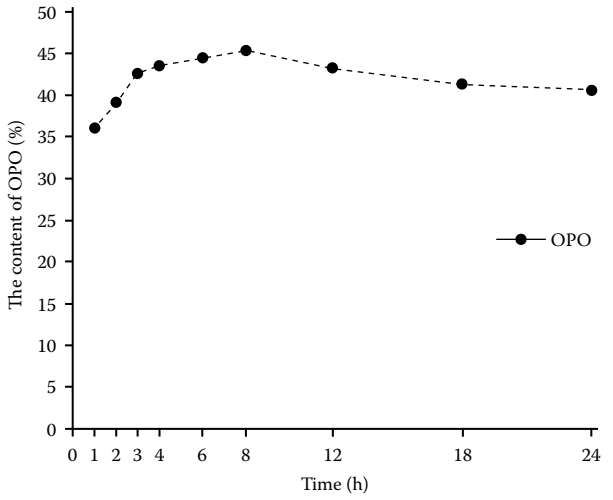

Figure 8. Effect of reaction time on 1,3-dioleoyl-2-palmitate triglyceride (OPO) content

CONCLUSION

In this paper, the synthesis of OPO, an important structural ester in human milk fat, was studied. The precursor materials for OPO synthesis were prepared by IM-ANL-catalysed hydrolysis, followed by OPO production via IM-ANL-mediated acidolysis. The enzyme is versatile, the production cost is economical, and the reaction conditions are optimised. According to the experimental results, the optimal reaction conditions were obtained: the amount of the enzyme in the hydrolysis of IM-ANL was 3%, the re-

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action temperature was 45 °C, and the reaction time was 48 h. The amount of the enzyme in the acidolysis interesterification of IM-ANL was carried out with 4%, 1 : 8 molar ratio of substrate PPP and OA, 55 °C reaction temperature, and 8 h reaction time. This study provided a more economical process for OPO synthesis. Instead of using commercial lipase, the laboratory-made IM-ANL was used, which not only reduced the cost of the enzyme, but also improved the use value of high-oleic vegetable oil. In the whole process, the reaction temperature is appropriate, which reduces the production cost and energy consumption, and helps to further promote the industrialization of OPO.

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