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Cocoa butter equivalent from enzymatic interesterification of tea seed oil and fatty acid methyl esters

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Abstract

Cocoa butter equivalent (CBE) was prepared by interesterification of tea seed oil, methyl palmitate and methyl stearate with lipase. The lipase was immobilized on macroporous resin selected from eight carriers. The rate of reaction of lipase immobilized on macroporous resin was 6.9 times higher than that of the free enzyme. After repeating application five times, 83.50% activity, of the immobilized lipase, was retained. Factors such as reaction time, temperature, water content, enzyme load and substrate ratio were studied. Three major acyls (palmitoyl, oleoyl and stearoyl) in triacylglycerols of the product were similar to those of cocoa butter. The melting range and dilatation–temperature curves of the prepared CBE were close to that of the cocoa butter.

1. Introduction

Cocoa butter is an important raw material in the chocolate and related confectionery industries because of its unique physical characteristics resulting from its unusual triacylglycerol composition. Its major components are saturated–unsaturated–saturated (SUS) triacylglycerols, which make cocoa butter hard and brittle at room temperature while melting completely in the human mouth. But limited supply and large demand lead to a quite high price. Cocoa butter equivalent (CBE) with a triacylglycerol composition similar to cocoa butter is prepared from lower value fats and oils. Several methods have been reported for the preparation of CBE. For example, the major triacylglycerol components of cocoa butter have been obtained by chemical interesterification (Tanaka, Irinatsu, Noguchi, & Kobayashi, 1987). Palm oil has been fractionated by acetone crystallization to yield a cocoa butter-like fraction (Ronald & Bexley, 1961). Recently, preparation of CBE through 1,3-specific lipase-catalyzed interesterification has received much attention because lipases offer certain advantages over other chemical catalysts. One of these advantages is that it produces less by-products. While chemical catalysts will randomize all of the fatty acids in a triacylglycerol mixture, 1,3-specific lipase can incorporate fatty acids into the sn-1,3-positions without changing the fatty acid residues in the sn-2-position. Other advantages are lower energy consumption and better product control. In order to improve the reuse and stability of lipase, immobilization of the lipase is necessary. Among several methods to immobilize lipase (Bagi & Simon, 1999; Gulay & Yasemin, 2002; Moreno, Arroyo, Hernáiz, & Sinisterra, 1997; Mojovic, Knezevic, Popadic, & Jovanovic, 1998; Zorica & Lopes, 1998), adsorption is considered to be most economical because the lipase is less inactivated during the operation as compared to other methods. In addition, the lipase immobilized by adsorption is also stable during interesterification because lipase is insoluble in organic

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solvent. There are many factors which influence the performance of immobilized lipase. One of the most important factors is the support material that affects both activity and stability of immobilized lipase.

In this study, lipase was immobilized on a macroporous resin selected from eight kinds of support materials. CBE was prepared by interesterification of tea seed oil, methyl stearate and methyl palmitate with the immobilized lipase. Several factors, such as reaction time, temperature, water content, enzyme load and substrate ratio were studied.

2. Experimental procedures

2.1. Materials

Porcine pancreas lipase (EC. 3.1.1.3) was purchased from Sigma Chemical Co. (St. Louis, MO). Tea seed oil was obtained from Shanghai Oil and Fat Co. (Shanghai, China). Methyl stearate, methyl palmitate, active carbon, silica gel, Celite, porous glass bead, macroporous resin, zeolite, florisil and 202 pink support were purchased from Shanghai Chemical Reagent Co. (Shanghai, China).

2.2. Lipase immobilization

Lipase was immobilized on the macroporous resin by adsorption. An appropriate amount of support (6.0 g) was mixed thoroughly with 1.2 g of lipase in 100 ml phosphate buffer (pH 7.5) for 4 h. The mixture of immobilized lipase was dried in a vacuum at room temperature until the water content of immobilized lipase was about 20%. The immobilized lipase was then stored at 4 °C

2.3. Interesterification reaction

The interesterification of methyl stearate (1.2 g), methyl palmitate (5.24 g) and tea seed oil (2.24 g) catalyzed by immobilized lipase (1.2 g) was carried out in an Erlenmeyer flask (50 ml volume) with a magnetic stirrer at 35 °C for 60 h.

2.4. Analytical methods

The course of the lipase-catalyzed interesterification, i.e., the substrate conversion, was followed by the determination of the stearic acid and palmitic acid content incorporated in the triacylglycerols of tea seed oil. The triacylglycerols were isolated from the reaction mixture by the TLC method (Paquot, 1979). The separated triacylglycerols were converted to fatty acid methyl ester with sodium methylate. The acyl composition was determined by a gas chromatograph (GC 7890F, TECHCOMP Co., Shanghai, China) fitted with a capillary column (AT SE-54, 15 m × 0.25 mm, 0.33 μm thickness) and equipped with FID. The temperatures of injection and column were 260 and 180 °C, respectively.

2.5. Determination of the dilatation of fats (Paquot, 1979)

The dilatometer bubble was filled with 5 g of fat. The dilatometer was weighed to determine the mass of fat both before and after filling. The dilatometer was then transferred to a water bath and maintained at 26 °C for 40 h, after which it was immersed in crushed ice water for 90 min and placed in a water bath maintained at temperatures between 25 and 45 °C. After 60 min, the level of the meniscus was read.

2.6. Analysis of the sterospecificity of CBE

The method used is the method described by Han (1995).

3. Results and discussion

3.1. Support materials screening

As shown in Table 1, the support materials have important influence on the activity of immobilized lipase. Eight supports were chosen for investigation of the interesterification activity of the immobilized lipase. In order to obtain a simple measure of the reaction rate on different supports, a substrate mixture consisting of tea seed oil and methyl stearate was used in this experiment. The reaction rate was calculated by measuring the incorporation of stearate into triacylglycerols of tea seed oil per hour, catalyzed by 1 g of immobilized lipase. The specific reaction rate was defined as the reaction rate per 1 mg of lipase. The result shows that the adsorption procedure used does not inactivate the lipase. The lipase immobilized on macroporous resin produced the highest reaction rate, specific reaction rate and immobilization efficiency among the eight supports. The specific reaction rate of immobilized lipase is 6.9 times higher than that of free lipase. The lipophilicity of support obviously affects the specific activity of immobilized lipase. The lipophilic matrix (crosslinked-polystyrene) of the macroporous resin attracts the substrate oil to either the support or the lipase surface so that tea seed oil concentration around the immobilized lipase is higher than that around the free lipase. As a result, the specific reaction rate produced by immobilized lipase on macroporous resin is greatly enhanced. In addition, the macroporous structure of resin makes lipase immobilization more efficient. The pores of the macroporous resin are 30 nm in diameter, which is about four times wider than

Table 1
The rate of interesterification on eight supports

Support material	Specific reaction rate (μ mol/mg prot h)	Reaction rate (μ mol/g h)	Immobilization efficiency (%)
Free lipase	0.063	21.5	–
Active carbon	0.128	8.70	33.98
202 pink support	0.058	3.14	27.22
Zeolite	0.040	2.03	25.59
Macroporous resin	0.437	78.7	90.5
Forisil	0.034	1.15	17.01
Silica gel	0.027	0.793	14.79
Porous glass bead	0.050	0.059	5.92
Celite	0.094	4.28	22.67

Reaction: Interesterification of tea seed oil (2.5 mmol) and methyl stearate (10 mmol). 0.5 g of lipase immobilized on different supports was added to reaction mixture. The reaction was carried out at 35 °C for 24 h.

lipase, so the lipase can enter the pores of macroporous resin more easily than it enters pores of porous glass beads which have a diameter of 2 nm (Goderis, 1990). For this reason, the immobilization efficiency of macroporous resin is 15.29 times higher than that of porous glass beads.

3.2. Operational stability of lipase immobilized on macroporous resin

The interesterification was catalyzed by immobilized lipase with different water content. As shown in Fig. 1, the enzyme activity retention of immobilized lipase with 10% water was 83.50% after five batches, whereas immobilized lipase with 40% water only retained 19.37% of its activity after five batches. Because water makes the conformation of protein more changeable, it exerts an important influence on enzyme inactivation. The porcine pancreas lipase (crude) used contains amylase and protease activity, which can hydrolyze the lipase at high water content.

3.3. Optimization of the interesterification conditions

In lipase-catalyzed interesterification, hydrolysis and esterification take place separately. As a reactant in the hydrolysis step and a product in the esterification step, water shifts the enzymatic interesterification equilibrium. In addition, water is necessary for enzyme activation. On the other hand, high water content will make the lipase unstable and lead to higher production of by-products such as diacylglycerols and fatty acids. In general, the water content is specific to a certain system, e.g., the particular lipase, support and solvent. As shown in Fig. 2, the optimum water content for palmitoyl was different from that of stearoyl. As the water content increased, palmitoyl incorporation increased. Palmitoyl incorporation reached its peak when the water content

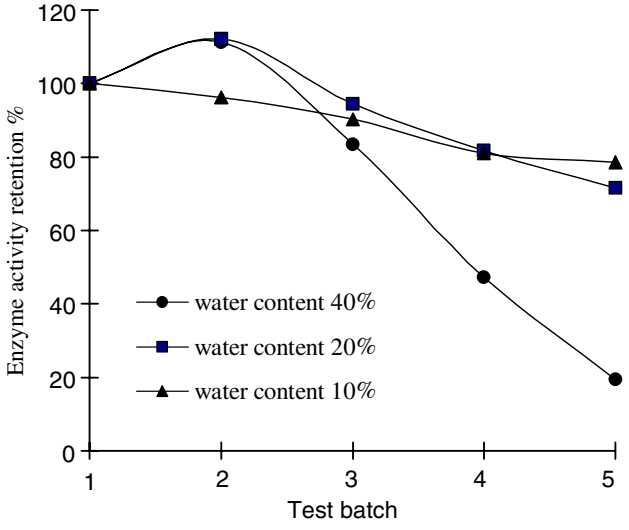

Fig. 1. Effect of water content on the operational stability of lipase immobilized on macroporous resin at 40 °C for 48 h. The graph represents the mean of two experiments.

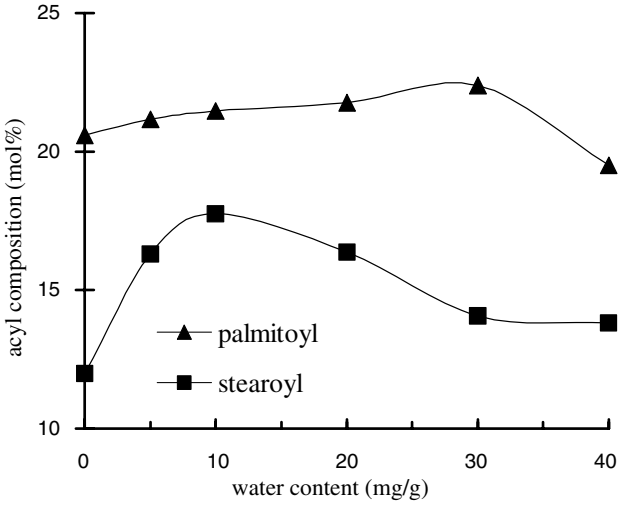

Fig. 2. Effect of water content on the percentage of the acyl groups of the interesterification reaction at 40 °C for 24 h. The graph represents the mean of two experiments.

was 30 mg/g, but there was no significant difference when the water content was between 10 mg/g and 30 mg/mg. Stearoyl incorporation reached its peak when the water content was 10 mg/g. To avoid producing by-products, we chose 10 mg/g water content in further CBE preparation.

3.4. Effect of the reaction temperature

Temperature also exerts an important influence on enzymatic interesterification. As shown in Fig. 3, the optimum temperature for interesterification was 30–40 °C. With an increase of reaction temperature, in the range of 40–60 °C, the acyl incorporation decreased significantly. There are several possible reasons to explain this phenomemon: (1) substrates, i.e., methyl stearate, can be well dissolved in hexane at 30–40 °C and lead to a low viscosity reaction mixture in which interesterification can be carried out quickly; (2) activation energy of the interesterification reaction is 30.4 J/mol (Goderis, 1990), which is so low that the reaction can be carried out efficiently at a low reaction temperature; (3) high temperatures inactivate the lipase, which leads to a strong decrease in reaction rate when the temperature is beyond 40 °C. The temperature of 35 °C was adopted in further CBE fat preparation.

3.5. Effect of substrate ratio

A higher methyl stearate and methyl palmitate concentration of reaction mixture favors product formation by providing excess acyl groups during interesterification. As shown in Fig. 4, a higher mole ratio of acyl donors yielded higher acyl incorporation. However, when the substrate ratio (tea seed oil:methyl stearate:methyl palmitate, mol/mol/mol) reached 1:6:6, there was no significant increase in incorporation. We chosed the substrate ratio of 1:8:8 for further CBE preparation.

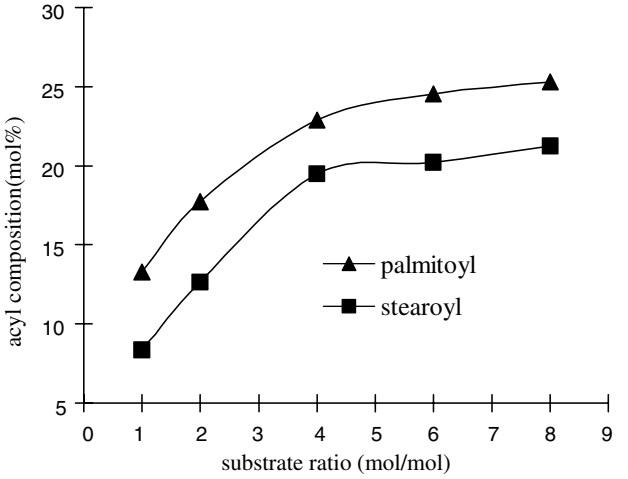

Fig. 4. Effect of the reaction substrate ratio on the interesterification reaction. The graph represents the mean of two experiments.

3.6. Effect of amount of the immobilized lipase added

The amount of immobilized lipase added was related to the reaction rate. Increased enzyme load will accelerate the reaction rate and improve the incorporation of acyl donors in interesterification under certain conditions. As shown in Fig. 5, the relationship between the acyl incorporation and the amount of added lipase was not linear. Increasing the amount of lipase above 25 wt% had no significant effect on substrate conversion.

3.7. Preparation of CBE

CBE was prepared by interesterification of tea seed oil with methyl stearate and methyl palmitate. The reac-

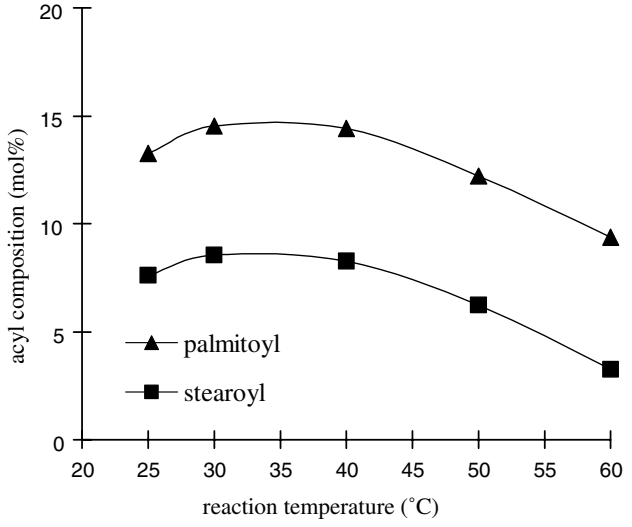

Fig. 3. Effect of the reaction temperature on the interesterification reaction for 12 h. The graph represents the mean of two experiments.

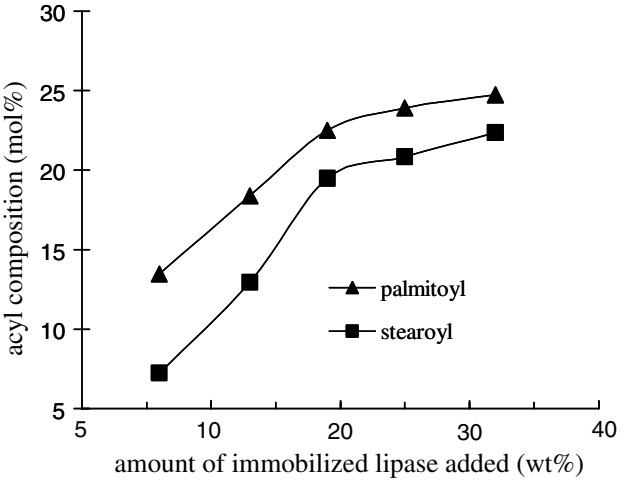

Fig. 5. Effect of reaction temperature on the interesterification reaction. The graph represents the mean of two experiments.

Table 2
Acyl composition of triacylglycerols of CBE, cocoa butter and tea seed oil

	Palmitoyl (mol%)	Oleoyl (mol)	Stearoyl (mol%)	Melting range
Tea seed oil	8.26	81.60	2.06	−9 to −5
Cocoa butter	31.54	33.27	32.19	34.2–36.8
CBE	31.43	35.30	29.26	33.3–37.7

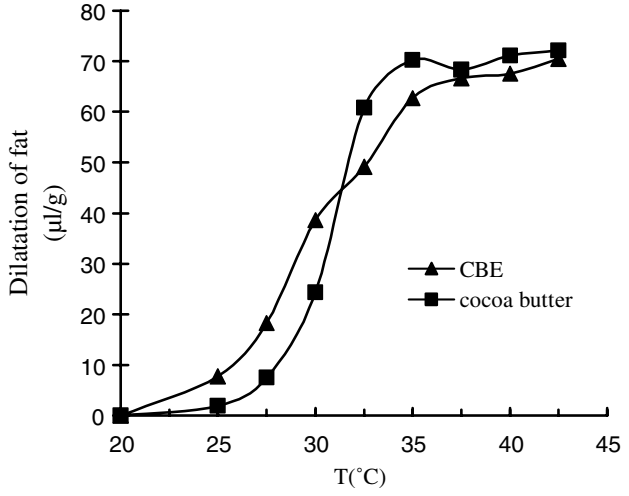

Fig. 6. The curve of the dilatation vs. the temperature change of the CBE and the cocoa butter. The graph represents the mean of two experiments.

tion condition was: substrate ratio, 1:8:8 (tea seed oil:-methyl stearate:methyl palmitate, mol/mol/mol); water content, 10 mg/g; reaction temperature, 35 °C; amount of immobilized lipase added, 15 wt%. The acyl composition of triacylglycerols in the reaction mixture was determined every 5 h during interesterification until it was similar to that of cocoa butter. The total reaction time was 60 h. The three major acyls in triacylglycerols of the product prepared are similar to those of cocoa butter (Table 2). Triacylglycerols of the product contain more oleoyl than of cocoa butter, which gives the product a lower initial melting point. The dilatation of cocoa butter and the product is shown in Fig. 6. The curve of CBE is similar to that of the cocoa butter, but the melting range is slightly wider. The acyls in sn-2 position of triacylglycerols of CBE analyzed by our laboratory are 77.19% oleoyl, 11.68% palmitoyl and 10.35% stearoyl (Han, 1995). This fact indicates that the product is composed mostly of SUS triacylglycerols, which are the main components of cocoa butter. The yield of CBE prepared in our experiment is 25.6% based on tea seed oil used.

Oleic acid accounts for about 74–87% of the triacylglycerols in tea seed oil at the sn-2 position (Sonntag, 1979). This means, tea seed oil is an ideal material for synthesis of CBE because pancreatic lipase prefers to interesterificate at sn-1 and 3 positions, acyl groups at sn-2 position remain the same. Therefore, when tea seed oil interesterifies with the mixture of methyl stearate and palmitate, catalyzed by pancreatic lipase, stearoyl–oleoyl–palmitin, stearoyl–oleoyl–stearin, polmitoyl–oleoyl–palmitin are the main products formed, and these are the main components of cocoa butter. Actually, CBE prepared here has a very similar dilatation curve and melting range to that cocoa butter as demonstrated in Fig. 6. Tea seed oil is an ideal material for commercial preparation of CBE.

Acknowledgement

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