Enzymatic Interesterification of Soybean Oil and Methyl Stearate Blends Using Lipase Immobilized on Magnetic Fe₃O₄/SBA-15 Composites as a Biocatalyst

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1 INTRODUCTION

Interesterification and hydrogenation are the main chemical processes available to improve the physicochemical properties of food lipids. For example, it is desirable to produce a semi-solid fat with good plasticity, tractility and shortening property, which can be achieved by partial hydrogenation of vegetable oils[1,2]. However, the hydrogenated product has higher amounts of trans fatty acids (FAs) that are detrimental and pose a threat to human health. Trans FAs have been reported to be able to increase low-density lipoproteins (LDL) and decrease high-density lipoproteins (HDL) in human blood, thus leading to several health problems such as increased risk factors of coronary artery disease[2,3]. Due to these adverse effects on health, the Food and Agriculture Organization (FAO) and World Health Organization (WHO) recommended that intake of trans FA should not exceed 4% and trans FA content of food should be reduced below this level. Accordingly, considerable efforts have been made to find suitable alternative methods to yield trans-free modified oils for different food applications. Recently, interesterification (IE), which alters the distribution of FAs in triacylglycerols (TAGs), has received much attention in the edible oil industry as a promising method for the modification of edible oils, since there is no possibility for trans FAs formation[4,5]. The interesterification between a high melting fat and liquid oil could result in the exchange of FA residues within and between TAGs with the formation of new altered TAG molecules, thereby producing semi-solid fats with desired physical and nutritional properties[6,7]. As a result, the interesterification has been considered as one of the feasible ways of obtaining low-trans or trans-free modified fats with great potential applications[8,9].

In general, interesterification can be achieved through either enzymatically or chemically catalyzed processes. The chemical catalysts, such as sodium metal and sodium alkoxide, are the most widely used homogeneous catalysts[10], with the advantage of cheap, easy to control and short reaction time. However, they are difficult to be separated from the reaction mixture, and the undesirable wastewater is produced in the downstream purification processes. These shortcomings have limited their applica-
tions in the food industry\textsuperscript{11,12}. Advantages of lipases versus classical chemical catalysts are that they operate in milder reaction conditions with fewer side reactions and avoid using hazardous toxic chemicals. Unfortunately, the practical application of an enzyme for the interesterification reaction is often hampered primarily owing to its high cost, the lack of long-term stability under processing conditions, the impossibility of multiple reuse in industrial processes, and the difficulty in its separation, recycling and reusing\textsuperscript{13}.

Many attempts have been made to overcome the aforementioned drawbacks of the free lipases. Among them, enzyme immobilization is a widely employed method to impart the desirable features of enzymes\textsuperscript{14,15}. Immobilized lipase provides important advantages to the process, such as the ease in catalyst recycling, continuous operation, and product purification. Apart from this, the immobilized lipase may be stabilized against denaturing agents that promote unfolding processes that can destroy the active sites\textsuperscript{16}. For the supports of immobilized enzyme, mesoporous materials, in particular SBA-15 silica, have received growing attention as promising supports for the enzyme immobilization because of their noteworthy properties, such as exceptionally high surface area, controlled pore size, and sufficient surface silanol groups for surface modification\textsuperscript{17,18}. In addition, considering the facile and fast separation of immobilized lipase, magnetic nanoparticles are also employed as supports for enzyme immobilization. Over the last decade, a variety of magnetic nanoparticles with immobilized enzymes have been adopted in several areas of application particularly for organic synthesis and bio-separation\textsuperscript{19}. The use of magnetic nanoparticles as supports for enzyme immobilization is based on the magnetic feature that the solid phase can achieve a rapid separation by using a magnetic field, and thereby leading to the decrease of operation cost\textsuperscript{20}. Therefore, the mesoporous SBA-15 materials modified with magnetite composites are excellent materials for the application of enzyme immobilization. To achieve this, the magnetic particles are attached on the surface of SBA-15 materials, and the abundant silanol groups can act as attachment sites for the immobilization of lipase by using glutaraldehyde as a coupling reagent.

Interesterification of edible oil usually involves at least two oils that have different FA compositions, in which the FA residues are distributed among the various positions on the glycerol backbone. In the current study, soybean oil and methyl stearate were employed as reactants to simplify the interpretation of the interesterification reaction, since the methyl stearate can act as a general and useful model compound for the interesterification of complex TAGs. The Fe\textsubscript{3}O\textsubscript{4}/SBA-15 composites were first prepared, and then amine-functionalized using 3-aminopropytriethoxysiane. The lipase from Candida rugosa was covalently immobilized onto the amino-functionalized Fe\textsubscript{3}O\textsubscript{4}/SBA-15 composites by using glutaraldehyde as a coupling reagent. Subsequently, the structure and magnetic properties of the magnetite Fe\textsubscript{3}O\textsubscript{4}/SBA-15 composites before and after lipase immobilization were characterized by using vibrating-sample magnetometer (VSM), and X-ray powder diffraction (XRD) techniques. The successful immobilization of lipase was confirmed by enzyme activity assays, and Fourier transform infrared (FT-IR) spectra. The activity of the biocatalyst for the enzymatic interesterification of soybean oil and methyl stearate was investigated regarding the incorporation of stearic acid into TAGs. The effects of various reaction parameters on the enzymatic interesterification were also evaluated in the present investigation.

2 EXPERIMENTAL

2.1 Materials

Candida rugosa lipase, Phuronic copolymer P123(EO\textsubscript{20}PO\textsubscript{70}EO\textsubscript{20}, average molecular weight of 5800), 3-aminopropytriethoxysiane, glutaraldehyde, and tetraethylorthosilicate (TEOS, 98\%) were obtained from Sigma-Aldrich. Methyl stearate was purchased from Shanghai Jinchung limited Corporation (Shanghai, China). Soybean oil, with an average molecular weight of 874 g mol\textsuperscript{-1}, was purchased from a local oil company. All other chemicals and solvents were of analytical or chromatographical grade.

2.2 Preparation of Fe\textsubscript{3}O\textsubscript{4}/SBA-15 Composites

Magnetic Fe\textsubscript{3}O\textsubscript{4} nanoparticles were readily prepared by the conventional co-precipitation method\textsuperscript{21}. In a typical procedure, 4.2 g of FeSO\textsubscript{4}·7H\textsubscript{2}O and 8.2 g of FeCl\textsubscript{3}·6H\textsubscript{2}O were firstly dissolved in 100 mL deionized water. When the solution was heated to 25°C, NH\textsubscript{4}·H\textsubscript{2}O solution was added dropwise under nitrogen atmosphere to the solution with vigorous stirring, leading to the formation of a dark suspension. Thereafter, the resultant slurry was aged in the mother solution at 80°C for 30 min, and the magnetite precipitates were magnetically decanted, and washed several times with distilled water and ethanol. Finally, the magnetite precipitates were dried in a vacuum oven at room temperature and stored for future use.

Mesoporous SBA-15 was prepared following the previously reported method\textsuperscript{22}. 5.0 g of Phuronic P123(EO\textsubscript{20}PO\textsubscript{70}EO\textsubscript{20}) was added to a mixture of 38 mL H\textsubscript{2}O and 150 mL 2 mol/L HCl aqueous solutions, which was stirred at 40°C for about 6 h until a clear solution was obtained. Then, 10.5 g of TEOS was added to this solution and continued to stir for 24 h, subsequently crystallized in a Teflon-lined autoclave at 100°C for 48 h. After crystallization, the solid products were collected by filtration, washed with water, dried at room temperature, and finally calcined at 550°C in flowing air.

SBA-15 (5.0 g) and Fe\textsubscript{3}O\textsubscript{4} magnetite (2.5 g) were im-
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mersed in acetic acid (25 mL) under mechanical stirring at room temperature. Afterwards, 25 mL of liquid paraffin, 3.5 mL of petroleum ether and 3.5 mL of Tween-60 were added to the solution. The resulting mixture was heated to 70°C, and 0.5 mL glutaraldehyde was then added and continued to stir vigorously for 3 h. After this, the solid precipitate was separated by using a magnetic field and washed thoroughly with petroleum ether, acetone and water, respectively, and finally dried at 60°C. The Fe₃O₄/SBA-15 composites thus obtained were black in color and showed a strong response to an external magnetic field.

2.3 Lipase Immobilization

The obtained magnetic Fe₃O₄/SBA-15 composite was used for the immobilization of lipase. To modify the Fe₃O₄/SBA-15 materials with amino groups, 1.0 g of Fe₃O₄/SBA-15 material was immersed in 20 mL of toluene, followed by addition of 0.4 g 3-aminopropyltriethoxysilane to the solution. The coupling reaction was carried out at a refluxing temperature for 12 h. After the reaction had finished, the prepared sample was collected by applying a magnetic field, washed with ethanol for several times, and dried at 60°C for 24 h under vacuum.

Lipase was covalently immobilized onto the amino-functionalized Fe₃O₄/SBA-15 composites by a typical glutaraldehyde activation procedure. 1.6 g of the amino-functionalized Fe₃O₄/SBA-15 material was submerged into a solution of native lipase from Candida rugosa (0.8 g) composed of 10% glutaraldehyde water solution (30 mL) and 20 mL of buffer solution (0.1 mol L⁻¹ phosphate buffer, pH 7.0). The immobilization process was carried out at 35°C in a shaking water bath for 12 h. The immobilized lipase composites were recovered by magnetic separation, washed with buffer solution (0.1 mol L⁻¹ phosphate buffer, pH 7.0), and freeze-dried for future use.

2.4 Characterization of the Immobilized Lipase

The KBr pellet technique was applied for determining the FT-IR spectra of the samples. Spectra were recorded on a Shimadzu IR-Prestige-21 spectrometer with 4 cm⁻¹ resolution. The scanning range was from 4000 to 400 cm⁻¹. Powder X-ray diffraction patterns were obtained on a Rigaku D/max-3B X-ray diffractometer (Tokyo, Japan) employing Cu Kα radiation (λ = 0.154 nm). The magnetic properties of the samples were determined with a LakeShore model 7304 vibrating sample magnetometer (VSM) at room temperature.

2.5 Enzymatic Interesterification Procedures

A blend of soybean oil and methyl stearate was charged into a 50 mL round-bottom flask. The blend was melted and homogenized at 60°C for 30 min. After lowering the temperature to 45°C, 15 wt% of immobilized lipase was added as a catalyst to the reactants. The enzymatic interesterification reaction between soybean oil and methyl stearate was carried out in the constant temperature bath oscillator at 45°C for 72 h. The reactors were sealed to minimize oxidation during the process. Once the reaction was completed, the immobilized lipase was recovered by magnetic separation, and the product was stored at 4°C for future analysis.

2.6 FA Composition Analysis

The formed TAGs were isolated from the reaction mixture by thin-layer chromatography (TLC) method. After interesterification reactions, the product mixture was spotted on TLC silica gel plates, and developed with petroleum ether/ethyl ether/acetic acid (90:10:1, v/v/v). Then, TLC plates were air-dried and sprayed with 0.2% 2,7-di-chlorofluorescein in methanol and visualized under UV light. The bands corresponding to TAGs, which were identified using triolein as the standard, were scraped from the plates and extracted with n-hexane. FA profile of the product was determined by a gas chromatography (GC) according to AOAC method. In brief, when the FA residues in TAGs had been completely converted into their corresponding fatty acid methyl esters (FAMEs), the formed methyl ester (0.2 μL) was injected into a Hewlett-Packard 7890 series (Agilent Technologies, Santa Clara, CA, U.S.A.). A gas chromatograph equipped with a flame-ionization detector was employed for the determination of FA composition using a fused-silica capillary column. The injector, detector and column temperatures were set at 260, 300 and 160°C, respectively. The FAME composition was identified by comparing the retention time of the sample peaks with respective FAME standards. The quantitative FA determination was conducted by area normalization and expressed as mass percent. All samples were analyzed in triplicate and the reported values are the average of three determinations.

2.7 Fatty Acid Composition at Sn-2 Position

The FA profile at the Sn-2 position of TAGs was determined by using pancreatic lipase in accordance with the method described in the literature. The pancreatic lipase can be applied to hydrolyze selectively the TAGs and can release FAs from the Sn-1,3 positions of TAGs. Briefly, 10 mg of TAGs and an appropriate amount of pancreatic lipase were mixed with 1 mL of Tris-HCl buffer (1 mol/L, pH 7.6), 0.25 mL of 0.05% bile salt solution, and 0.1 mL of 2.2% calcium chloride solution. The sample was incubated in a water bath at 37°C for 3 min, vortexed for 2 min. To quench the reaction, 1 mL of 6 mol/L HCl solution and 4 mL of diethyl ether were added, vortexed for 2 min, and centrifuged at 1000 rpm for 3 min. The diethyl ether layer was passed through the anhydrous sodium sulfate column, and the volume was subsequently reduced under nitrogen to obtain the concentrated sample. Next, the concentrated
samples were spotted on TLC plates, which were coated with silica gel G, and placed in the tank and then developed with a solvent system of hexane/diethyl ether/formic acid mixture (70:30:1, v/v/v). The plates were sprayed with 0.2% 2,7-dichlorofluorescein in methanol, visualized under UV light, and 2-monocacylglycerol (2-MAG) bands were identified with 2-oleoylglycerol as the standard. After that, the bands corresponding to 2-MAG were scraped off, extracted with 5 mL ethyl ether, and then methylated and analyzed by GC techniques for the determination of FA composition at Sn-2 position.

3 RESULTS AND DISCUSSION

3.1 Characteristics of the Immobilized Lipase

The Fe₃O₄/SBA-15 composite has high concentrations of hydroxyl groups on its framework surface, and these hydroxyl groups can act as immobilizing sites for lipase by using glutaraldehyde as cross-linking reagent. Thus, lipase can be covalently immobilized on the magnetic particles by forming a Schiff base linkage between the aldehyde group of glutaraldehyde and the terminal amino group of lipase. The immobilized lipase shows good susceptibility to a magnetic field, and thereby could be easily separated by magnetic decantation.

The proof binding of lipase onto the magnetic particles was confirmed by FT-IR spectrum. Figure 1 shows FT-IR spectra of SBA-15 (a), free lipase (b), amino-functionalized Fe₃O₄/SBA-15 composite (c), and immobilized lipase composite (d). As can be clearly seen from Fig. 1, three characteristic absorption bands at 1086 cm⁻¹, 805 cm⁻¹, and 468 cm⁻¹ were observed for SBA-15 silica. These IR bands were ascribed to the Si-O-Si vibration. Besides, a broad band at about 3400 cm⁻¹ could be attributed to the surface OH stretching vibration, and a characteristic band in the mid-infrared region at 947 cm⁻¹ for SBA-15 silica was mainly due to the bending vibration of Si-OH group in SBA-15 silica (Fig. 1, spectrum a)²⁰. For the pure lipase, the IR absorption peaks of 1654 cm⁻¹ and 1541 cm⁻¹ were characteristics peaks of lipase (Fig. 1, spectrum b). In the case of amino-functionalized Fe₃O₄/SBA-15 composite (Fig. 1, spectrum c), the peaks located at 3370 cm⁻¹ and 1630 cm⁻¹ were principally ascribed to the stretching and bending vibrations of NH₂, respectively. After immobilization of the lipase on the magnetic particles (Fig. 1, spectrum d), the IR bands responsible for the lipase that was chemically covalent-bonded to the magnetic particles, were observed at 1636 cm⁻¹ for amide I and at 1549 cm⁻¹ for amide II.²⁷ In addition, the IR peak appeared at 1662 cm⁻¹ could be assignable to stretching vibrations of C=N bond, indicating the formation of schiff base as a result of the reaction between carbonyl group of glutaraldehyde and amine group of lipase²⁸. The presence of Fe₃O₄ magnetite could be confirmed by the strong absorption band at 574 cm⁻¹, which corresponded to the Fe-O stretching vibrations of Fe₃O₄²⁹. Given the IR results, the lipase is indeed attached to the surface of magnetic composites successfully.

The Fe₃O₄ magnetite, Fe₃O₄/SBA-15 composite and immobilized lipase composite were characterized by XRD techniques. As indicated in Fig. 2, for the Fe₃O₄ particles, six diffraction peaks of Fe₃O₄ recorded at 2θ of 30.1°, 35.5°, 43.1°, 53.4°, 57.0°, and 62.6°, could be assigned to the (220), (311), (400), (422), (511), and (440) planes, respectively. These XRD peaks revealed that the magnetite particles were pure Fe₃O₄ with a spinel crystal structure (JCPDS database file, No.79-0418)³⁰. For Fe₃O₄/SBA-15 compos-
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ites, the same characteristic XRD peaks were also observed. When the lipase was covalently bound to the magnetic composites, the sample had XRD patterns identical to that of Fe₃O₄ particles. This result showed that the binding of lipase could not change the spinel structure of Fe₃O₄ that is essential for retention of magnetic properties. As a consequence, the immobilized lipase could preserve the magnetic behavior, which is beneficial for application in separation processes.

The magnetic properties of Fe₃O₄ magnetite, Fe₃O₄/SBA-15 composite and immobilized lipase composite were evaluated using a VSM technique at room temperature. According to the results in Fig. 3, the saturation magnetization of pure Fe₃O₄ magnetite was found to be 76.4 emu/g, while the saturation magnetization of Fe₃O₄/SBA-15 composite was decreased to 62.3 emu/g owing to the existence of non-magnetic SBA-15 silica. Accordingly, the magnetic support can be separated from the reaction mixture easily and rapidly due to the large saturation magnetization. The saturation magnetization of the immobilized lipase composite was determined to be 57.8 emu/g. Obviously, the Fe₃O₄/SBA-15 composite and immobilized lipase composite had small values of the saturation magnetization when compared with Fe₃O₄ magnetite, mostly due to an increase in the surface anisotropy induced by SBA-15 silica. Besides, the magnetization curve exhibited zero remanence and coercivity, which is indicative of the superparamagnetization of the particles 31. These magnetic properties of the immobilized lipase can render them very susceptible to a magnetic field and thus make the solid and liquid phases separate easily.

3.2 The Immobilized Lipase-Catalyzed the Interesterification Reaction

The application of magnetic particles as a support for the lipase immobilization has many advantages in comparison to the free enzyme. The immobilized lipase can be easily recovered from the reaction mixture, which is of interest for the separation process. Besides, the immobilization of lipase can increase the stability of lipase and maintain the good catalytic activity.

The total FA profile and the positional distribution of the FA residues along the glycerol backbone in the interesterified oils were determined and the results are presented in Table 1. Soybean oil typically had major FA compositions of 55.2 % linoleic acid, 22.2 % oleic acid, and 11.9 % palmitic acid, with small amounts of linolenic acid (7.5 %) and stearic acid (3.4 %). No interesterification reaction occurred without catalyst under the reaction conditions employed here. After the interesterification by using the immobilized lipase, the modified oil mainly contained 17.5 % linoleic acid, 15.4 % oleic acid, 12.7 % palmitic acid, 2.2 % linolenic acid, and 52.2 % stearic acid. Clearly, the content of stearic acid in the TAGs was significantly increased after the enzymatic interesterification reaction. As the methyl stearate used in this study contained a small amount of methyl palmitate (7.8 %), a slight increase in the content of palmitic acid after the interesterification was also observed. As can be seen from Table 1, the Sn-2 position of soybean oil mainly constituted 22.6 % oleic acid, 69.1 % linoleic

![Fig. 3](image)

**Fig. 3** Room temperature magnetization curves for Fe₃O₄ magnetite (a), Fe₃O₄/SBA-15 composite (b), immobilized lipase composite (c).

<table>
<thead>
<tr>
<th>FA †</th>
<th>Total FA composition</th>
<th>FA composition of sn-2 position</th>
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<tbody>
<tr>
<td></td>
<td>before IE ‡</td>
<td>after IE ‡</td>
</tr>
<tr>
<td>Palmitic acid (16:0)</td>
<td>11.9</td>
<td>12.7</td>
</tr>
<tr>
<td>Stearic acid (18:0)</td>
<td>3.4</td>
<td>52.2</td>
</tr>
<tr>
<td>Oleic acid (18:1)</td>
<td>22.2</td>
<td>15.4</td>
</tr>
<tr>
<td>Linoleic acid (18:2)</td>
<td>55.2</td>
<td>17.5</td>
</tr>
<tr>
<td>Linolenic acid (18:3)</td>
<td>7.5</td>
<td>2.2</td>
</tr>
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</table>

† Fatty acid; ‡ Interesterification
acid, 1.3% palmitic acid, 0.8% stearic acid, and 6.2% linolenic acid. However, the FA profile at Sn-2 position of the interesterified oil was greatly varied after the interesterification for oleic acid to 19.4%, for linoleic acid to 33.7%, for palmitic acid to 11.0%, for stearic acid to 32.5%, for linolenic acid to 33.7% and for linolenic acid to 3.4%. The obvious change of FA moiety at Sn-2 position is mainly owing to the enzymatic interesterification with methyl stearate. Besides, no trans FAs were detected in the interesterified oils catalyzed by the immobilized lipase. The interesterification does not cause isomerization in the interesterified product. Therefore, the results outlined in Table 1 showed the enzymatic interesterification between soybean oil and methyl stearate was indeed occurred by using the immobilized lipase.

3.3 Influence of Enzymatic Interesterification Parameters

3.3.1 Influence of reaction temperature

In the bio-catalytic process, the operating conditions are investigated to optimize the interesterification procedure. The quantification of the amount of stearoyl incorporation in the TAGs was carried out by using GC methods. Table 2 shows the stearoyl incorporation into the TAGs by the enzymatic interesterification reaction at various temperatures, ranging from 35°C to 55°C. In the absence of any solvent, the reaction temperature needs to be raised above the melting point of methyl stearate, so that all species in the reactants are in liquid state. As indicated in Table 2, the change in reaction temperature significantly altered the stearoyl incorporation into the interesterified TAGs. When the temperature was below 45°C, the stearoyl incorporation into the interesterified TAGs was increased with increasing the reaction temperature. The highest incorporation of stearic acid occurred at a reaction temperature of 45°C. However, upon further increasing the reaction temperature beyond 45°C, the catalytic activity of the bound lipase was shown to be decreased, with the reduced stearoyl incorporation at higher temperature than 45°C, mostly due to the negative effect of the temperature increase on enzyme stability. Generally, higher reaction temperature not only can favor endothermic reaction because of the shift in thermodynamic equilibrium, but also can lead to the deactivation of lipase. Based on the temperature study, the suitable temperature for the interesterification catalyzed by the immobilized lipase is 45°C.

3.3.2 Influence of substrate ratio

The substrate ratio is one of the important variables affecting the interesterification reaction. The influence of substrate molar ratio on the interesterification reaction is illustrated in Table 2. As can be seen, the incorporation of stearic acid into the interesterified TAGs was considerably increased from 10.2% to 52.2% by increasing the substrate molar ratio from 0.5:1 to 1.5:1. The maximal incorporation degree of stearic acid was attained at the methyl stearate to soybean oil molar ratio of 1.5:1. However, the further increase in the substrate molar ratio beyond 1.5:1 gave a drop in the stearoyl incorporation. By drawing on the results, the appropriate substrate molar ratio is chosen at 1.5:1.

3.3.3 Influence of reaction time

Table 2 also displays the stearoyl incorporation as a function of reaction time. As the reaction time increased...
from 36 to 72 h, the incorporation of stearic acid, catalyzed by the immobilized lipase, appeared to be increased from 14.1% to 52.2%. The highest stearoyl incorporation was found at a reaction time of 72 h. The further increase in reaction time beyond 72 h, did not improve the incorporation of stearic acid. Therefore, the reaction time required to approach reaction equilibrium for the enzymatic interesterification is essentially 72 h.

3.3.4 Reusability of the Immobilized Lipase

The stability and reusability of the immobilized lipase, is of great importance from viewpoint of the practical application. The immobilized lipase was recovered by magnetic separation, washed three times with phosphate buffer (0.1 mol L⁻¹ phosphate buffer, pH 7.0), rinsed with tert-butanol, and then used again for next interesterification cycle. The each assay conditions remained the same as described above. It was shown that the stearoyl incorporation into the interesterified TAGs was 52.2%, 51.5%, 48.6% and 45.8% as the immobilized lipase was recycled for 1, 2, 3, and 4 times, respectively. Thus, the immobilized lipase could be reused for the interesterification reaction for four times without significant loss of its catalytic activity. However, a significant decrease in the stearoyl incorporation to 31.8% was observed after five cycles of reuse, probably resulted from the denaturation and by-product inhibition. In view of the results, the immobilized lipase has a better stability and can be used for four cycles.

4 CONCLUSIONS

The amino-functionalized magnetic Fe₃O₄/SBA-15 composites were successfully prepared using 3-aminopropyltriethoxysiane, and then employed to immobilize lipase from Candida rugosa by using glutaraldehyde as a coupling reagent. The immobilized lipase was used as an environmentally benign biocatalyst for the interesterification of soybean oil and methyl stearate. The experimental results indicated that the immobilized lipase had the potential for catalyzing the interesterification reaction. The immobilized lipase could be separated easily and used for four cycles.

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