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Lipase-catalyzed Synthesis of Starch Palmitate in Mixed Ionic Liquids

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ABSTRACT: Lipase-catalyzed synthesis of starch palmitate was investigated in ionic liquid mixtures consisting of 1-butyl-3-methyl-imidazolium acetic ([BMIm]Ac) and 1-butyl-3-methyl-imidazolium tetraflouroborate ([BMIm][BF_4]). The effect of reaction parameters such as the amount of enzyme used, the reaction temperature, the mass ratio of [BMIm][BF_4]/[BMIm]Ac, the molar ratio of methyl palmitate/anhydroglucose unit in starch, and the reaction time on the degree of substitution was studied. The formation of starch esters was confirmed by the presence of the carbonyl signal in the FT-IR and NMR spectra. Scanning electron microscopy and X-ray diffraction data showed that the morphology and crystallinity of starch esters were largely disrupted. Thermogravimetric analysis suggested thermal stability of starch palmitate decreased compared to native starch. Water contact angle measurements revealed that the hydrophobicity of starch esters was improved after modification. The successful lipase-catalyzed synthesis of starch palmitate in ionic liquids suggested that ionic liquids could be used as a potentially attractive green alternative to harmful organic solvents for synthesis of high fatty acid starch ester.

KEYWORDS: *starch palmitate, ionic liquids, lipase, synthesis*

INTRODUCTION

As a cheap, abundant, and renewable natural material, starch has been chemically modified to improve its properties for many years. Various kinds of starch derivatives have been investigated, among which high fatty acid starch ester is one of the most important kinds. High fatty acid starch ester has special thermoplasticity, hydrophobicity, and biodegradability properties. They can serve as an internal plasticizer,[1] and their potential utilization in drug delivery systems and other biomedical applications has been also investigated. [2] High fatty acid starch ester is commonly prepared by reacting starch with fatty acid chlorides, fatty acid vinyl esters, or carboxylic acids dispersed in organic solvents such as dimethylsulfoxide (DMSO), pyridine, toluene, or *N*,*N*-dimethyl acetamide (DMAC)/LiCl.[3−7] However, the volatile, toxic organic solvents used and the harsh reaction conditions required resulted in environmental and safety problems, which limited commercial development of this technology. Therefore, there is a strong incentive to develop an environmentally benign process for the synthesis of high fatty acid starch ester. Recent works have described the production of fatty acid starch esters with a broad range of degree of substitution (DS) values (0.01−0.31) using fatty acid vinyl or methyl ester or fatty acid anhydride, with supercritical carbon dioxide as the solvent.[8] However, the experimental equipments and techniques used in these methods are relatively sophisticated .

Because of unique properties, such as low melting point, low or negligible vapor pressure, nonflammability, and recyclability, room-temperature ionic liquids (ILs) have been considered as possible green replacements for organic solvents.[9,10] Progress has been achieved in applying ILs into starch chemistry in recent years. Some ILs (containing Cl^-, Ac^-, NO_3^- anions) have been found to be capable of dissolving starch.[11−15] Moreover, several kinds of starch esters such as starch acetate, starch succinate, starch phosphate have been chemically synthesized using ILs as reaction media in recent years.[16−18] However, research on synthesis of high fatty acid starch ester in ILs has rarely been reported. Only Xie and Wang[19] performed the homogeneous synthesis of high fatty acid esters of corn starch (starch laurate and starch stearate) in 1-butyl-3-methylimidazolium chloride ([BMIm]Cl). The achieved maximal DS values of starch laurate and starch stearate were 0.37 and 0.28, respectively. However, the toxic pyridine had been still utilized as a catalyst during the reaction process. Compared with general chemical methods, biocatalysis provided a greener route with wide applications. Producing esterified carbohydrates through enzyme catalysis in ILs is currently strongly investigated. Lee et al.[20] described Novozyme 435-catalyzed synthesis of 6-*O*-lauroyl-D-glucose in ionic liquid mixtures. Park and Kazlauskas[21] reported that lipase-catalyzed acetylation of glucose proceeded with higher regioselectivity compared to reactions in conventional solvents.

Recently, literature on lipase-mediated esterification of carbohydrates with high fatty acid in ILs is mainly focusing on synthesis of sugar fatty acid esters. Research on biotransformation of disaccharides or polysaccharides in ILs is much less documented. The major reason is that extremely hydrophilic ILs, such as nitrate anion ILs, which have great starch dissolving potential, seem to inactivate biocatalysts. Lipases showed no activity even in some less hydrophilic (acetate, dicyanamide (DCA)) ionic liquids.[14,22] Meanwhile, starch is only sparingly soluble in ILs, such as [BMIm][BF_4], which are a benign reaction medium for biocatalysis.[11,23] To search for an appropriate reaction system that could combine the hydrophobicity of the acyl donor, the hydrophilicity of starch, and the lipase's ability to function at satisfactory levels, we investigated the lipase-catalyzed synthesis of high fatty acid starch ester in IL mixtures consisting of [BMIm]Ac and [BMIm][BF_4] in this work. Modification conditions, including

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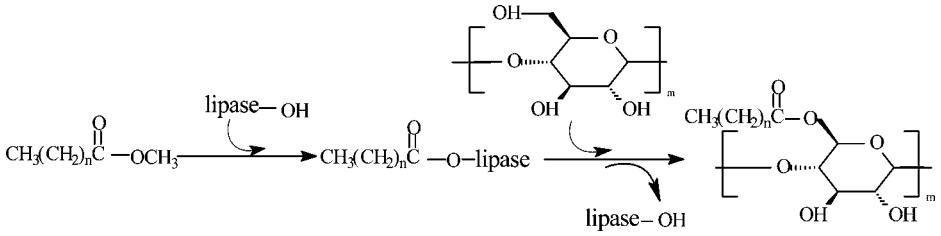

Figure 1. Lipase-catalyzed esterification reaction of starch carried out in ionic liquids.

reaction temperature, reaction time, amount of enzyme, mass ratio of [BMIm][BF_4]/[BMIm]Ac, and molar ratio of methyl palmitate/anhydroglucose unit (AGU) in starch, were investigated. High fatty acid starch esters were also characterized by Fourier transform infrared (FT-IR) spectroscopy, ^{1}H NMR spectroscopy, scanning electron microscopy (SEM), X-ray diffractometry (XRD), and thermogravimetric analysis (TGA). This work may provide a green pathway to prepare a high fatty acid starch ester by replacing traditional organic solvents with ionic liquids.

MATERIALS AND METHODS

Materials. High-amylose maize (Hylon VII) starch was purchased from National Starch LLC (Bridgewater, NJ, USA) and dried at 50 °C for 24 h before use. 1-Butyl-3-methyl-imidazolium acetic ([BMIm]Ac, >99%) and 1-butyl-3-methyl-imidazolium tetraflouroborate ([BMIm]-[BF_4], >99%) were obtained from Lanzhou Institute of Chemical Physics (Lanzhou, China). Methyl palmitate (>97%) was purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). All other chemicals were of analytical grade.

The enzyme Candida rugosa lipase (E.C. 3.1.1.3.) (nominal activity: 739 U/mg enzyme) from *Candida rugosa*, type VII was obtained from Sigma–Aldrich (Shanghai, China). The enzyme activity was determined by the method of olive oil hydrolysis.[24] The released fatty acids were determined by titration with 5 mM NaOH in ethanol. One unit of enzyme activity was defined as the amount of lipase that liberates 1 μmol of fatty acids per minute under the assay condition.

Dissolution of Starch in IL Mixtures. Dried starch (1.62 g) was added into 24 g of mixed ionic liquids ([BMIm][BF_4]/[BMIm]Ac) in a three-neck round flask, which was continuously purged with gaseous N_2. The mass ratio of [BMIm][BF_4]/[BMIm]Ac was studied according to the required conditions. The mixture was stirred for homogeneous mixing and heated in an oil bath at 120 °C for 2 h.

Lipase-catalyzed Synthesis of High Fatty Acid Starch Esters. After the complete dissolution of starch in ILs, the mixture was cooled to the required reaction temperature. Methyl palmitate and lipase were added according to the conditions required by the experimental design and then stirred at the desired temperature for a certain amount of time. After the completion of the reaction, the reaction mixture was cooled to room temperature, and high fatty acid starch esters were subsequently precipitated with anhydrous ethanol under vigorous stirring followed by centrifugation. The precipitate was washed thoroughly with sufficient anhydrous ethanol to eliminate the residual IL, unreacted reagents, and byproduct. Finally, the solid was filtered and dried in vacuum at 40 °C for 48 h. The products obtained were used for testing.

Determination of DS. The DS value of modified starch is defined as the average number of substituent of hydroxyl groups per D-glucopyranosyl structural unit of the biopolymer. DS values of starch esters were determined according to the reported titration method. [25] One gram of dry starch ester was accurately weighed and dispersed in 50 mL of water containing 10 mL of 0.5 mol/L NaOH solution followed by vigorously stirring at room temperature for 4 h. Excess NaOH was then back-titrated with 0.5 mol/L HCl solution using phenolphthalein as an indicator. The DS value of starch ester was calculated by using the following equation:

$$\mathrm{DS} = 162M(V_0 - V)/1000W \quad (1)$$

where 162 is the molecular weight of the AGU, V_0 is the volume in milliliters of the 0.5 mol/L HCl solution used for titrating the blank, V is the volume in milliliters of the 0.5 mol/L HCl solution used for titrating the sample, M is the exact molarity of the used HCl solution, and W is the sample weight in grams as dry substance.

Characterization of High Fatty Acid Starch Esters. FT-IR spectra of native starch and starch esters were acquired on a Nicolet 510 spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA) using KBr disk technique. For FT-IR measurement, the samples were mixed with anhydrous KBr and then compressed into thin disk-shaped pellets. The spectra were obtained with a resolution of 2 cm^{-1} and a wavenumber range of 400–4000 cm^{-1}. ^{1}H NMR spectra were recorded on an Bruker 600 MHz (Bruker Corporation, Fallanden, Switzerland). The sample was dissolved in deuterated dimethyl sulfoxide (DMSO-d_6) at 30 °C. The spectra were obtained at 30 °C with a pulse angle of 30°, a delay time of 10 s, and an acquisition time of 2 s. All chemical shifts were reported in parts per million (ppm). XRD patterns were recorded on a RU200R X-ray diffractometer (Rigaku, Tokyo, Japan) operating at 40 mA and 40 kV by using Cu Kα filtered radiation (λ = 0.154 nm). The scattering angle (2θ) was varied from 5° to 45° with a step width of 0.04°. SEM images of samples were examined by means of a model 1530VP scanning electron microscope (LEO, Oberkochen, Germany). The accelerating voltage was 20 kV. The samples were mounted on an aluminum stub with a double sticky tape followed by coating with the gold in a vacuum before examination. TGAs were completed with a Diamond TG-DTA thermogravimetric analyzer (PerkinElmer Co., Waltham, MA, USA). The apparatus was continually flushed with nitrogen. About 10 mg of samples was heated from 60 to 650 °C with a heating rate of 10 °C/min. Water contact angle measurements were carried out by the sessile drop method (at 20 °C) using a OCA40 microscope–goniometer system (Dataphysics Instruments, Stuttgart, Germany). The starch ester films were prepared according to a literature procedure.[26] A drop (1.5 μL) of water was placed on a freshly prepared film using a precision microsyringe. After 30 s, the contact angle was measured at least at six different places on the film. The reported values were the average of the independent measurements.

RESULTS AND DISCUSSION

Due to the semicrystalline structure of starch granules, the reagents cannot readily penetrate into the granules, which results in a low DS value in traditional heterogeneous conditions. However, the emergence of homogeneous chemical modification of starch in various ionic liquids has opened up an avenue for exploring new end uses. As described in Figure 1, lipases could catalyze the esterification of starch reaction by first forming a covalently linked, highly reactive lipase–FAME intermediate, which could then react with the hydroxyl group of the anhydroglucose units of starch to form high fatty acid starch ester.

Effect of ILs. The acylation of starch catalyzed by lipase in IL mixtures is obviously a complex reaction system with many factors at play. [BMIm]Ac is able to destroy the semicrystalline

structure of native starch granules by disrupting hydrogen bonding between hydroxyl groups in starch molecules. However, it breaks intramolecular associations in the lipase molecules and reduces the activity of lipase. [BMIm][BF_4] is a benign medium for biocatalysis reaction, while it cannot dissolve starch. Hence, there must be a compromise between enzymatic activity and starch solubility for the suitable reaction medium.

The effect of ILs on DS is presented in Table 1. The DS increased with an increase in the mass ratio of [BMIm][BF_4]/[BMIm]Ac. The DS value reached 0.144 when the mass ratio of [BMIm][BF_4]/[BMIm]Ac was 19:5. However, the decrease in DS was observed with increasing mass ratio beyond 19:5. The reason was that the amount of [BMIm]Ac in IL mixtures was not sufficient enough for the complete dissolution of starch granules. For the reaction media with mass ratio of [BMIm]-[BF_4]/[BMIm]Ac 20:4, we tried to dissolve the starch by prolonging the dissolving time from 2 to 4 h during the dissolution process. However, the solution was still turbid because of insoluble starch granules. The DS value declined sharply from 0.144 to 0.034 when the mass ratio changed from 19:5 to 17:7. This was due to the negative influence of [BMIm]Ac on lipase molecules. It should be noted that these DS values were much higher as compared to the corresponding values in the previous report that the acquired maximum DS value of product was 0.0195 when lipase-coupling esterification of starch with octenyl succinic anhydride (OSA) was studied in aqueous phase.[27] These comparable data suggested that lipase-catalyzed esterification of starch was very efficient in ionic liquid system.

Table 1. Effect of Mass Ratio of Ionic Liquids on the DS[a]

mass ratio of [BMIm][BF_4]/[BMIm]Ac	20:4	19:5	18:6	17:7
DS	0.121 ± 0.002 c	0.144 ± 0.004 d	0.061 ± 0.005 b	0.034 ± 0.003 a

[a]Other reaction conditions: methyl palmitate/AGU, 3:1; lipase dosage, 0.1 g; reaction temperature, 60 °C; reaction time, 3 h. Values in the same row with different superscript letter are significantly different ($p < 0.05$).

Effect of Molar Ratio of Methyl Palmitate/AGU. The data in Table 2 indicated the effect of the molar ratio of methyl palmitate/AGU on the DS value of the product. It showed that the DS value of starch palmitate rose steadily when the molar ratio of methyl palmitate/AGU increased from 1 to 3. However, if the molar ratio was enhanced further, a slight reduction in DS value was observed. It may be explained that, when the increased amount of methyl palmitate was added, the concentration of lipase in the reaction system would be depleted. Therefore, the optimal molar ratio of methyl palmitate/AGU was 3:1, when the DS value of starch palmitate reached 0.144. The similar trend was also observed by Xie and Wang[19] who attempted to synthesize fatty acid starch ester in IL homogeneous medium.

Table 2. Effect of Molar Ratio of Methyl Palmitate/AGU on the DS[a]

molar ratio of methyl palmitate/AGU	1:1	2:1	3:1	4:1
DS	0.068 ± 0.002 a	0.100 ± 0.006 b	0.144 ± 0.004 d	0.136 ± 0.003 c

[a]Other reaction conditions: [BMIm][BF_4]/[BMIm]Ac, 19:5; lipase dosage, 0.1 g; reaction temperature, 60 °C; reaction time, 3 h. Values in the same row with different superscript letter are significantly different ($p < 0.05$).

Effect of Lipase Dosage. The correlation between the lipase dosage and the DS value of starch palmitate is described in Table 3. An increase of lipase dosage from 0 to 0.05 and 0.1 g resulted in noticeable improvement of the DS of starch palmitates from 0.006 to 0.110 and 0.144, respectively. Apparently, lipase could significantly improve the efficiency of esterification of starch in the system. However, the DS value of starch palmitate was almost constant when lipase dosage was beyond 0.1 g, which suggested the influence of the excess amount of lipase on reaction was not significant under the given reaction conditions. As we know, lipase dosage is a very critical variable in biotransformation of starch. Organic solvents, such as DMSO and DMF, were widely used for esterification of starch. Qiao et al.[28] studied the effect of enzyme amount on the DS values of alkenyl ketene dimer (AKD)−starch in DMSO. They reported that the DS values of the AKD−starch increased from 0.004 to 0.012 with an increasing dosage of lipase PS from 8 to 24 wt % relative to starch. The achieved highest DS value was 0.012 when the lipase dosage was 24 wt%. However, DMSO is an extremely polar solvent, which may cause loss of catalytic activity in enzymes to some extent. Unlike organic solvents of comparable polarity, ionic liquids often do not inactivate enzymes,[21] which explains the comparatively higher DS value in our products. These data further confirmed that the ionic liquid system is a more benign medium than organic solvent for lipase-catalyzed esterification of starch.

Table 3. Effect of Lipase Dosage on the DS[a]

lipase dosage (g)	0	0.05	0.10	0.15	0.20
DS	0.006 ± 0.001 a	0.110 ± 0.002 b	0.144 ± 0.004 c	0.150 ± 0.005 c	0.153 ± 0.003 c

[a]Other reaction conditions: [BMIm][BF_4]/[BMIm]Ac, 19:5; methyl palmitate/AGU, 3:1; reaction temperature, 60 °C; reaction time, 3 h. Values in the same row with different superscript letter are significantly different ($p < 0.05$).

Effect of Reaction Temperature. The effect of the reaction temperature on the reaction process was investigated by setting different reaction temperatures (50, 60, 70, and 80 °C), and the results are shown in Table 4. Reaction temperature has an important effect on DS value of high fatty acid starch ester because it affects both the activity of lipase and the penetration speed of reagents. A rise of reaction temperature from 50 to 60 °C led to an increase in the DS of products from 0.105 to 0.144. However, DS value decreased sharply when the reaction temperature was beyond 60 °C. This phenomenon could be probably due to the inactivation of

Table 4. Effect of Reaction Temperature on the DS[a]

reaction temp (°C)	50	60	70	80
DS	0.105 ± 0.005 b	0.144 ± 0.004 c	0.074 ± 0.002 a	0.069 ± 0.003 a

[a]Other reaction conditions: [BMIm][BF_4]/[BMIm]Ac, 19:5; methyl palmitate/AGU, 3:1; lipase dosage, 0.1 g; reaction time, 3 h. Values in the same row with different superscript letter are significantly different ($p < 0.05$).

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lipase under high temperature, which was in line with the study of Xu et al.[27] When starch was modified by lipase-coupling esterification with octenyl succinic anhydride, increases in the reaction temperature from 30 to 40 °C resulted in increases in DS. However, DS values decreased with further increases in the reaction temperature from 40 to 70 °C.

Effect of Reaction Time. The effect of the reaction time on DS value is presented in Table 5. The DS value of starch palmitate increased from 0.053 to 0.144 as the reaction time was prolonged from 1 to 3 h. However, when the reaction lasted for more than 3 h, a slightly reduction of the DS value was observed. One reason for this phenomenon may be that the hydrolysis of starch palmitate occurred. Another reason is due to partial deactivation of the enzyme in reaction system because of the prolonged time. This observed phenomenon was consistent with the results on enzyme-catalyzed preparation of AKD-grafted starch studied by Qiao et al.[28]

Table 5. Effect of Reaction Time on the DS[a]

reaction time (h)	1	2	3	4
DS	0.053 ± 0.002 a	0.082 ± 0.006 b	0.144 ± 0.004 c	0.136 ± 0.003 bc

[a]Other reaction conditions: [BMIm][BF_4]/[BMIm]Ac, 19:5; methyl palmitate/AGU, 3:1; lipase dosage, 0.1 g; reaction temperature, 60 °C. Values in the same row with different superscript letter are significantly different ($p < 0.05$).

FT-IR Analysis. Figure 2 illustrates the FT-IR spectra of native starch (Figure 2a), starch palmitate with DS 0.068 (Figure 2b), and starch palmitate with DS 0.144 (Figure 2c). In the spectrum of native starch, the C−O bond stretching vibrations of AGU show several discernible absorbencies at 1155, 1081, and 1020 cm^{-1}. Meanwhile, the O−H stretching and the C−H stretching vibrations give strong signals at 3377 cm^{-1} and at 2930 cm^{-1}, respectively. [29] In comparison with native starch, FT-IR spectra of starch palmitates with different DS (Figure 2b, c) exhibited a new band at 1744 cm^{-1}, which was attributed to a carbonyl C═O symmetry deformation vibration. The new absorption indicated that the esterified starch products were formed during the esterification process. With the increase of DS, the intensities of the peak at 1744 cm^{-1} increased. Normally, for high fatty acid starch esters, there was a large increase in the intensity of the C−H stretching vibration at 2930 cm^{-1}.[7] However, in this work, due to the relatively low DS value of the products, there was only a minimal increase shown in Figure 2c. A similar phenomenon was also observed in previous literature.[30,31]

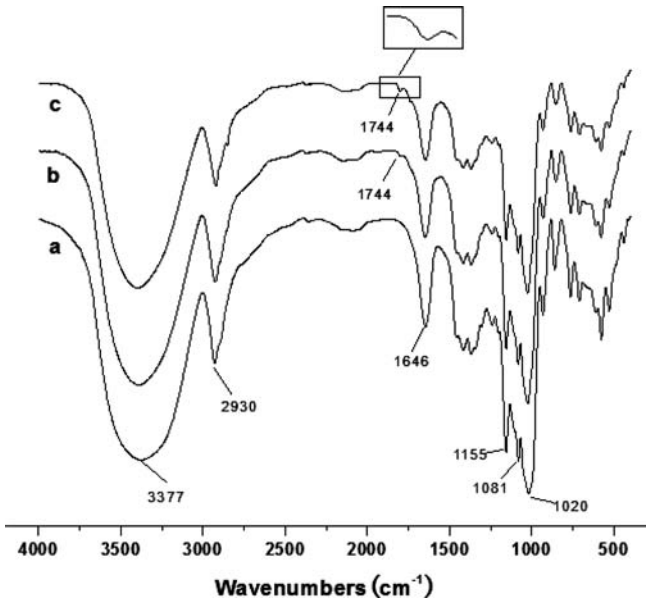

Figure 2. FT-IR spectra: (a) native starch; (b) starch palmitate (DS 0.068); (c) starch palmitate (DS 0.144).

^{1}H NMR Spectra Analysis. Figure 3 shows the typical ^{1}H NMR spectrum of native starch (Figure 3a), starch palmitate with DS 0.068 (Figure 3b), and starch palmitate with DS 0.144 (Figure 3c). ^{1}H chemical shifts of the protons at 3.64 ppm were assigned to H-3, 3.57 ppm to H-5, 3.31 ppm to H-2, and 3.15 ppm to H-4. The chemical shifts of H-1 and OH-2, 3, 6 were assigned to peaks between 4.58 and 5.50 ppm (Figure 3a).[32] With the esterification process, acyl groups substituted the hydrogen atom of the hydroxyl groups in the starch, which led to the change of proton resonances of the anhydroglucose unit. The ^{1}H NMR spectra of starch palmitate showed three protons of the terminal methyl group of the acyl chain around 0.8 ppm (peak 10 in Figure 3b, c). Peak 7 is related to the methylene group, beside the carbonyl group, and the one at 1.4 ppm (peak 8) is the methylene group directly before it. All the other methylene groups in the acyl chain have a peak at 1.2 ppm (peak 9 in Figure 3b, c).[7] Moreover, the intensity of those four peaks (peaks 7−10) increased with the increase of DS.

XRD Analysis. X-ray diffraction was performed to investigate the change of the crystallinity of native corn starch and starch palmitate (Figure 4). In the XRD pattern of native starch (Figure 4a), strong reflections (2θ) were found at about 5.5°, 17°, 22°, and 24°, which indicated that native starch exhibited a typical B-type X-ray pattern.[33] However, the peak characteristic of native B-type starch almost disappeared after modification in homogeneous conditions, which was caused by the disrupting of the inter- and intramolecular hydrogen bonds during the dissolving processes. Starch palmitate with DS 0.121 (Figure 4b) exhibited diffraction peaks at 13.5°and 20.7°. Starch palmitate with DS 0.144 (Figure 4c) showed diffraction peaks at 7.8° 13.5°,and 20.7°, lines characteristic of amylose V-type complexes, which could originate from a single-helical structure "inclusion complex" made up of starch molecules and ethanol.[34] These results indicated that the crystallinity pattern of starch palmitate was converted into V-type, which was in accordance with the findings of Geng et al.[35]

SEM Analysis. Scanning electron microscopy was used to investigate the morphology of native starch and starch palmitate. As can be seen from Figure 5a, b, native corn starch granules were round or oval shapes with various sizes. In comparison with the unmodified starch, starch palmitate exhibited a whole different morphology. As shown in Figure 5c−f, starch granules were completely disrupted and lost their individuality and smoothness. The change of granular structure could be mainly attributed to the dissolving process when the solvent ILs ([BMIm]Ac) disrupted the hydrogen bonds of starch and sequentially destroyed the crystalline structure of starch granules. Undoubtedly, it could significantly improve the efficiency of reaction of starch with lipase−FAME intermediate during the modification process.

Thermal Analysis. The thermal properties of native starch and starch palmitate are presented in Figure 6. As can be seen from the TG curves of native starch (Figure 6a), there was no

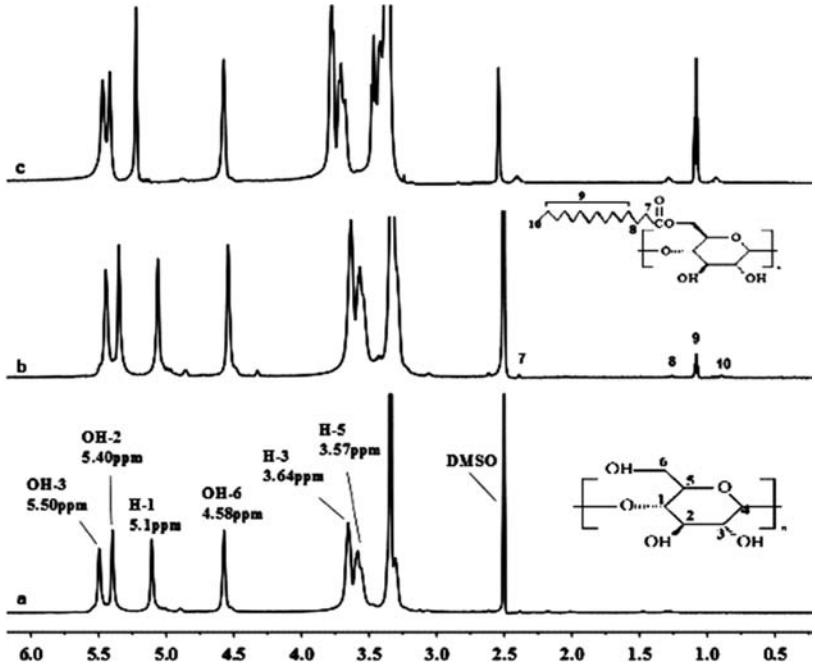

Figure 3. [1]H NMR spectra: (a) native starch; (b) starch palmitate (DS 0.068); (c) starch palmitate (DS 0.144).

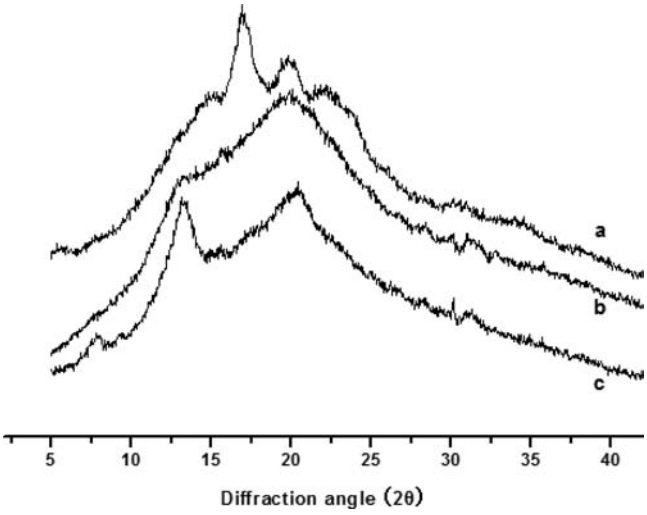

Figure 4. X-ray diffraction spectra: (a) native starch; (b) starch palmitate (DS 0.121); (c) starch palmitate (DS 0.144).

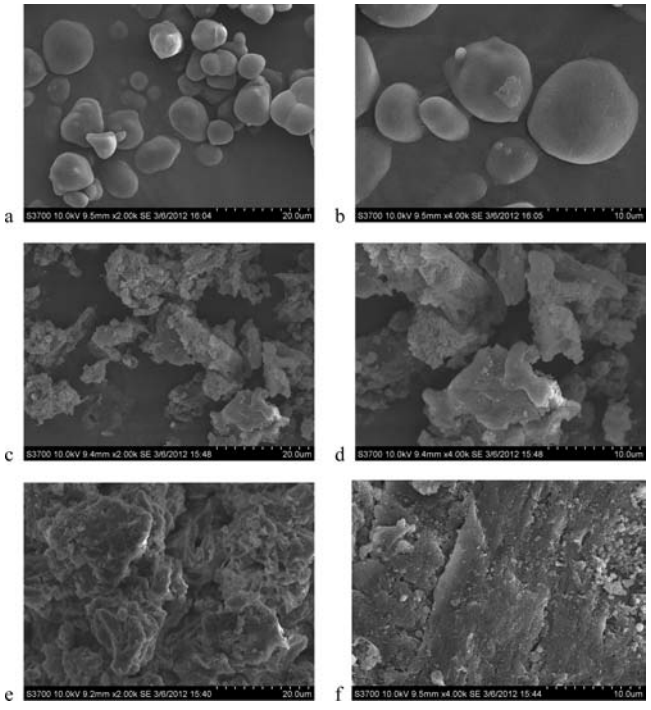

Figure 5. SEM images of starch: (a) native starch ×2000; (b) native starch ×4000; (c) starch palmitate ×2000; (d) starch palmitate ×4000 (DS 0.121); (e) starch palmitate ×2000; (f) starch palmitate ×4000 (DS 0.144).

weight loss until a temperature of 304 °C was reached. With further heating, it showed a two-stage weight loss below 600 °C. The DTG plot of native starch (Figure 6a) showed the first minor peak at 290 °C resulted from the decomposition of water, together with the other peak at 320 °C corresponding to the starch decomposition. In the case of starch palmitates (DS 0.121 and 0.144), decomposition commenced at 273 and 280 °C, respectively, with a steady loss continuing with an increase in temperature, until 600 °C (Figure 6b, c). Weight loss of water was disappeared in starch palmitates. The DTG plots (Figure 6b, c) exhibited one single peak for the degradation to carbonaceous residues,[1] which could be explained by the increased hydrophobicity of starch after esterification as the accessible OH groups in the ionic liquid homogeneous system were replaced by the hydrophobic hexadecyl groups. Furthermore, the observed degradation peak for native starch was 327 °C, whereas the values for starch palmitates were 307 and 310 °C, respectively. These results indicated that esterification of starch with high fatty acid in [BMIm][BF_4]/[BMIm]Ac homogeneous system reduced the initial temperature of their thermal degradation and the thermal stability compared to native starch. However, some previous reports have demonstrated that the thermal stability of starch was improved after esterification of starch with fatty acid in a heterogeneous system.[1,4] The observed differences could be

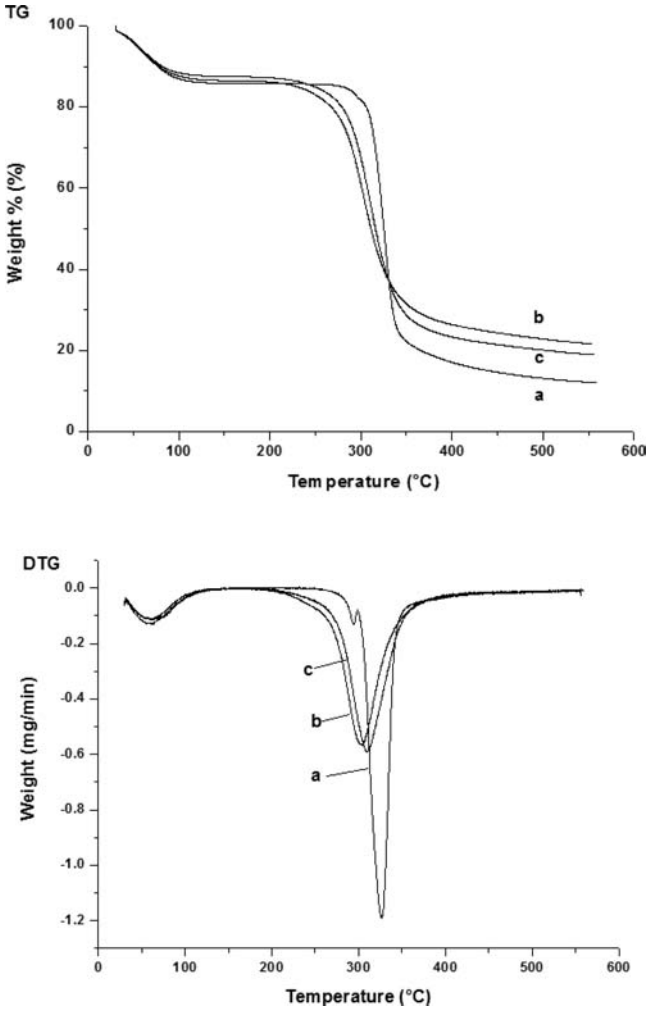

Figure 6. TG and DTG curves: (a) native starch; (b) starch palmitate (DS 0.121); (c) starch palmitate (DS 0.144).

due to the distinct modification mechanism of starch in the ionic liquid homogeneous system. The semicrystalline structure of starch granules was completely transformed into an amorphous structure during dissolution process. This explanation has been confirmed by the above XRD and SEM analyses.

Water Contact Angle Measurement. Contact angle measurements were performed on native starch and the starch palmitate samples with various DS values (0.034−0.144). The results are given in Table 6. Native starch had a contact angle of

Table 6. Hydrophobicity of Starch Palmitates[a]

DS	0	0.034	0.068	0.144
contact angle	46.2 ± 1.5 a	75.9 ± 1.7 b	80.6 ± 1.1 c	82.3 ±1.4 c

[a]Values in the same row with different superscript letter are significantly different ($p < 0.05$).

46.2°, which is in agreement with the reported value for native corn starch (43°).[36] All starch palmitate products show higher contact angles (75.9−82.3°) compared to native starch (46.2°), which is indicative for a higher hydrophobicity of the products compared to native starch.[37] These values are comparable to that of methyl methacrylate (PMMA), which is a hydrophobic synthetic polymer and has a contact angle of 83°.[38] As shown in Table 6, the hydrophobicity of starch palmitates increased with the increase of DS values. The increased hydrophobicity of esterified starch is attributed to the replacement of hydrophilic hydroxyls by the relatively hydrophobic ester groups.

This manuscript described an exploratory study on the lipase-catalyzed synthesis of high fatty acid starch ester using [BMIm][BF_4]/[BMIm]Ac mixtures as the solvent. A series of starch palmitates were prepared with DS values ranging from 0.034 to 0.153. The molecular structure of starch palmitate was confirmed by FT-IR and ^{1}H NMR spectroscopies. The structure of the starch granule was destroyed, and the B-type crystalline structure was lost after the modification reaction. Compared with native starch, the thermal stability of the high fatty acid starch ester decreased. The modified starches improved the hydrophobicity performance of starch materials, exhibiting good applied prospect. With respect to further research, the break through will lie in finding a new proper ionic liquid, which can overcome the problem of restricted starch solubility and low enzyme stability in various reactions.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

[BMIm]Ac, 1-butyl-3-methyl-limidazolium acetic; [BMIm]-[BF_4], 1-butyl-3-methyl-imidazolium tetraflouroborate; AGU, anhydroglucose unit; [BMIm]Cl, 1-butyl-3-methylimidazolium chloride; DMAC, *N*,*N*-dimethyl acetamide; DMSO, dimethylsulfoxide; ILs, ionic liquids; DS, degree of substitution; DCA, dicyanamide; FT-IR, Fourier transform infrared spectroscopy; XRD, X-ray diffraction; NMR, nuclear magnetic resonance; SEM, scanning electron microscopy; TGA, thermogravimetric analyze; FAME, fatty acid methyl ester; AKD, alkenyl ketene dimer; DMF, dimethyl formamide

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