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Two-Step Method of Enzymatic Synthesis of Starch Laurate in Ionic Liquids

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ABSTRACT: Enzymatic esterification of starch with long-chain fatty acid was investigated by using ionic liquids 1-butyl-3-methylimidazolium choride ([BMIm]Cl) and 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIm][BF_4]) as reaction media. An industrial lipase produced by *Candida rugosa* was used to modify starch with lauric acid. The effect of reaction parameters such as the lipase dosage, the molar ratio of lauric acid/anhydroglucose unit (AGU) in starch, and the reaction temperature as well as the reaction time on the degree of substitution (DS) of long-chain fatty acid starch esters was studied. The maximum DS value was 0.171 under the given conditions. The maximum solubility of high-amylose starch measured by turbidity was 11.0/100 g of [BMIm]Cl. The esterification products were confirmed according to Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR) analysis. The morphological and crystallographic properties of native starch were largely disrupted during modification process as indicated by scanning electron microscopy (SEM) and X-ray diffraction (XRD) data. The thermal stability of the starch laurates was found to decrease compared to native starch. After reaction, the ionic liquid was effectively recycled and reused. This paper explores the potential of ionic liquids as solvent for the enzymatic synthesis of long-chain fatty acid starch esters.

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

INTRODUCTION

Starch is nontoxic, renewable, biodegradable, and modifiable.[1] There has been increasing interest in manufacturing value-added products based on starch. Fatty acid starch ester is an important starch derivative, which is based on acylation reactions of free hydroxyl groups in the anhydroglucose unit (AGU). It has various potential medical and industrial applications, for example, utilization in drug delivery systems and substituting for oil-based plastic polymers, especially in packaging industries.[2,3]

The hydrophilic feature of native starch severely limits the development of starch-based materials.[4] Additionally, native starch often exists in granules, which result in low solubility in most of conventional solvents. Organic solvents including dimethyl sulfoxide (DMSO), *N*,*N*-dimethylacetamide (DMAC)/LiCl, or pyridine are used as reaction media for preparing fatty acid starch esters.[5−8] However, application of organic solvents has many disadvantages. The flammable, volatile, and toxic nature of these organic solvents makes the separation process dangerous and increases the risk of environmental pollution because of solvent loss. Regarded as the solvents of future, ionic liquids (ILs) have potential use in sustainable processes as solvent replacements due to their low toxicity, nonflammability, very low vapor pressures, and recyclability.[9] In the meantime, the influence of ILs on the environment has also been investigated, for example, the relationship between cation and anion structures of ILs and their toxicological properties.[10] Recently, research concerning the development of less toxic ILs was reported.[11]

ILs attract great attention for starch modification because some are found to be good solvents for starch. 1-Butyl-3-methylimidazolium choride ([BMIm]Cl) has been reported to be effective in dissolving starch. Stevenson et al.[12] compared the structures and molecular weights of four different origins of starch (corn, rice, wheat, and potato) dispersed in [BMIm]Cl and in deionized water. They showed that starch heat-dispersed in [BMIm]Cl aggregated into clumps of individual particles <1 μm in diameter, whereas the particle size of aggregates from starch dispersed in water was much larger, suggesting that starch is more soluble in [BMIm]Cl than in hot water. Recently, a series of starch esters, namely, acetate starch, succinate starch, and phosphate starch, have been synthesized in [BMIm]Cl through chemical routine.[13−15] However, these studies were mainly concentrated on the synthesis of short-chain starch esters (C1−C4), especially on starch acetate (C2). Long-chain fatty starch esters (C12, C18) were first synthesized in [BMIm]Cl with the catalysis of a toxic compound, pyridine.[16]

Lipases are a class of biocatalysts that catalyze esterification, ester hydrolysis, or transesterification processes. Some lipases are known to be very thermostable and active in organic solvent media.[17] Although esters can be synthesized chemically, the application of enzyme technology provides environmental advantages as well as a reduction in the consumption of energy. In the past decades, ILs have been increasingly exploited as solvents in a wide variety of biocatalyst reactions and processes.[18] They have shown advantages of common

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organic solvents for biocatalysis reactions. Moreover, many enzymes have exhibited higher selectivity, activity, or stability in some ILs than in organic solvents.[19] In recent years, application of ionic liquids in the synthetic biotransformation of carbohydrate has gained great attention. Several ILs containing $[BF_4]^-$, $[PF_6]^-$, and $[TfO]^-$ anions have been reported to be useful as reaction media for biotransformation of sugars.[20,21] However, the low solubility of starch in these ILs still limits the effective biotransformation of starch.

To combine the high solubility of starch and the lipase's catalysis ability in IL reaction system, we investigated an exploratory study on the lipase-catalyzed synthesis of long-chain fatty acid starch ester using IL mixtures consisting of [BMIm]Ac and [BMIm][BF_4] in our earlier research.[22] Native starch granules were destroyed and esterified in the same media. In this work, a two-step methodology of synthesizing long-chain fatty acid starch esters was investigated to further achieve good starch solubility compatible with high enzymatic activity in IL system by separating starch solubility from starch esterification. First, native starch was dissolved in the ionic liquid [BMIm]Cl to destroy the granular structure of starch, which would yield starch chains more accessible to other reactants and most importantly the bulky lipase molecule. Morphology and thermal properties of starch heat-dispersed in [BMIm]Cl were analyzed. In particular, turbidity was measured to determine the solubility of starch in [BMIm]Cl. Then the dissolved starch was esterified with lauric acid in 1-butyl-3-methylimidazolium tetraflouroborate ([BMIm][BF_4]) using *Candida rugosa* lipase (CRL) as catalyst. Selected systems were optimized with respect to reaction time, reaction temperature, lipase dosage, and molar ratio of lauric acid/AGU in starch. Products were also characterized by ^{1}H NMR spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA). Finally, the recycling and reuse of ILs are discussed.

MATERIALS AND METHODS

Materials. High-amylose maize (Hylon VII) starch was obtained from National Starch LLC (Bridgewater, NJ, USA) and dried at 50 °C for 24 h before use. [BMIm]Cl (>99%) and [BMIm][BF_4] (>99%) were purchased from Lanzhou Institute of Chemical Physics (Lanzhou, China).

The enzyme *Candida rugosa* lipase (EC 3.1.1.3.) from *C. rugosa*, type VII, was obtained from Sigma-Aldrich (Shanghai, China). PPL (porcine pancreatic lipase) and PS (*Pseudomonas fluorescens* lipase) were also purchased from Sigma-Aldrich. Other lipases, namely, lipase TLIM (*Thermomyces lanuginosus* lipase immobilized on silica gel) and Novozym 435 (*Candida antarctica* B lipase immobilized on a macroporous acrylic resin) were supplied by Novozymes A/S (Bagsvaerd, Denmark). The enzyme activity determination was based on the method of olive oil hydrolysis according to the published procedure.[23] The released free fatty acids were measured by titration with 5 mM NaOH in ethanol. One unit of enzyme activity (U) was defined as the amount of lipase that releases 1 μmol of titratable free fatty acids per minute under the described conditions. The activities of CRL, PPL, lipase PS, lipase TLIM, and Novozym 435 were detected as 739, 462, 685, 634, and 28 U/mg, respectively.

Turbidimetric Measurements. Turbidity measurement was performed according to the method of Luo et al.[24] A precise quantity of [BMIm]Cl (40 g) and a magnetic stirrer were placed in a turbidimetric glass vial and heated in an oil bath at 100 °C with stirring. A small precise amount of starch was added discretely into each vial. At least 30 min was allowed for dissolution after each addition. The turbidity of the IL/starch mixture was measured by a nephelometer (2100AN, Hach Co., Loveland, CO, USA) until it reached a stable nephelometric turbidity unit (NTU) value.

Gelatinization of Starch in IL. Dried starch was added to [BMIm]Cl at a concentration of 10% in a three-neck round flask. The mixture was placed in an oil bath and heated at 100 °C for 3 h with vigorous magnetic stirring under a N_2 atmosphere. After the mixture was cooled to room temperature, starch was isolated by precipitation with sufficient anhydrous ethanol and centrifugation at 7740*g* for 20 min. The solid product was then washed thoroughly with deionized water, followed by anhydrous ethanol to eliminate [BMIm]Cl. Finally, starch was collected by filtration and then dried under vacuum at 60 °C for 48 h. The prepared samples were used for testing and synthesis of long-chain fatty acid starch esters. In the meantime, all of the ethanol containing [BMIm]Cl was collected and recycled.

Lipase-Catalyzed Synthesis of Long-Chain Fatty Acid Starch Esters. Dried pregelatinized starch (1.62 g) was added into 16.2 g of [BMIm][BF_4] in a three-neck round flask. The mixture was heated with magnetic stirring at the required reaction temperature for about 1 h. Then lauric acid was added to the solution with the molar ratio of lauric acid/AGU in starch at 1:1, 2:1, 3:1, and 4:1. CRL (0, 0.15, 0.3, and 0.45 g) was added into the mixture. The mixture was stirred at the desired temperature of 50, 60, 70, or 80 °C for 3, 5, 7, 9, or 11 h. The reaction was stopped after the required time had elapsed. After the reaction mixture reached room temperature, the resulting products were precipitated with anhydrous ethanol, and long-chain fatty acid starch esters were separated by centrifugation. The solid was further washed with anhydrous ethanol. After filtration, the residue was dried in a vacuum oven at 40 °C for 48 h. All of the ethanol containing [BMIm][BF_4] was collected and recycled.

Recycling of ILs. At the end of each experiment, the ILs ([BMIm]Cl and [BMIm][BF_4]) were collected and recycled. The collected [BMIm]Cl and ethanol mixture from the gelatinization process was first filtered with a core funnel to remove solid particles. Then the filtrate containing [BMIm]Cl and ethanol was distilled by rotary evaporation at 40 °C to remove ethanol. The residual solution was further dried at 95 °C by rotary evaporation to evacuate the moisture. The collected [BMIm][BF_4] and ethanol mixture from the esterification process was first distilled by rotary evaporation at 40 °C until the ethanol was evaporated. Then the residual solution was filtered with a core funnel to remove the concretionary lauric acid and solid particles. The filtrate was further dried at 95 °C by rotary evaporation to evacuate the moisture. The purity was determined by ^{1}H NMR spectroscopy. The recycled ILs were reused to prepare long-chain fatty acid starch esters.

Determination of Degree of Substitution (DS). The DS of long-chain fatty acid starch esters was determined according to the published titration method.[25] One gram of accurately weighed starch ester was dispersed in 50 mL of water. Then 10 mL of 0.5 mol/L NaOH solution was added, and the solution was stirred at room temperature for 4 h. After 2−3 drops of phenolphthalein indicator was added, the solution was titrated against 0.5 mol/L HCl solution. The DS value of starch ester was calculated by using the equation

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

where 162 is the molecular mass of an AGU, V_0 is the volume of 0.5 mol/L HCl solution consumed when titrating the blank, V is the volume of 0.5 mol/L HCl solution consumed when titrating the sample, M is the molarity of standard HCl solution, and W is the exact weight of the dry sample analyzed.

Characterization of Pregelatinized Starch and Long-Chain Fatty Acid Starch Esters. The FT-IR spectra of native starch, dried pregelatinized starch, and starch esters were recorded from a KBr disk containing finely ground samples on a Nicolet 510 spectrophotometer (Thermo Electron Corp., Waltham, MA, USA) in the range of 400−4000 cm^{-1}. ^{1}H NMR spectra analysis was obtained from a Bruker 600 MHz (Bruker Corp., Fallanden, Switzerland). The starch and starch ester samples were dissolved in deuterated dimethyl sulfoxide (DMSO-d_6) at 30 °C. The ILs were dissolved in deuterium water (D_2O). Each spectrum was recorded at 30 °C, the delay time was 10 s, and the acquisition time was 2 s. XRD patterns were performed on an

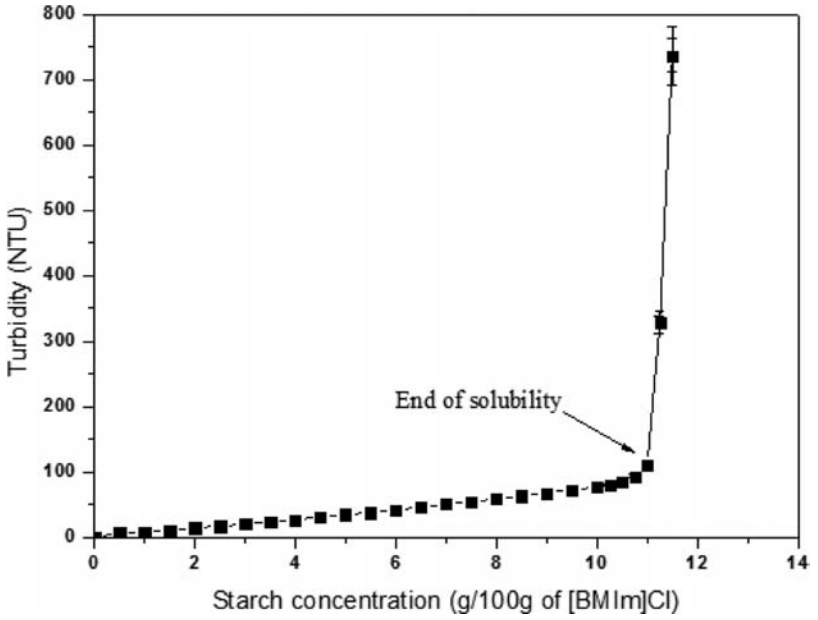

Figure 1. Turbidity of high-amylose maize starch dissolved in [BMIm]Cl at 100 °C.

Table 1. Effect of Lipase Dosage on the DS[a,b]

	lipase dosage			
	0 g	0.15 g	0.3 g	0.45 g
DS	0.004 ± 0.001a	0.085 ± 0.005b	0.165 ± 0.003c	0.171 ± 0.004d

[a]Other reaction conditions: lauric acid/AGU, 3:1; reaction temperature, 60 °C; reaction time, 5 h. [b]Values in the same row with different letters are significantly different ($p < 0.05$).

Table 2. Effect of Molar Ratio of Lauric Acid/AGU on the DS[a,b]

	molar ratio of lauric acid/AGU			
	1:1	2:1	3:1	4:1
DS	0.104 ± 0.004a	0.136 ± 0.006b	0.165 ± 0.003c	0.161 ± 0.002c

[a]Other reaction conditions: lipase dosage, 0.3 g; reaction temperature, 60 °C; reaction time, 5 h. [b]Values in the same row with different letters are significantly different ($p < 0.05$).

Table 3. Effect of Reaction Temperature on the DS[a,b]

	reaction temperature			
	50 °C	60 °C	70 °C	80 °C
DS	0.048 ± 0.004a	0.165 ± 0.003c	0.131 ± 0.002b	0.087 ± 0.005ab

[a]Other reaction conditions: lauric acid/AGU, 3:1; lipase dosage, 0.3 g; reaction time, 5 h. [b]Values in the same row with different letters are significantly different ($p < 0.05$).

Table 4. Effect of Reaction Time on the DS[a,b]

	reaction time				
	3 h	5 h	7 h	9 h	11 h
DS	0.102 ± 0.006a	0.165 ± 0.003c	0.163 ± 0.005c	0.153 ± 0.004b	0.147 ± 0.002b

[a]Other reaction conditions: lauric acid/AGU 3:1, lipase dosage 0.3 g, reaction temperature 60 °C. [b]Values in the same row with different superscript letter are significantly different ($p < 0.05$).

RU200R X-ray diffractometer (Rigaku, Tokyo, Japan). The diffraction patterns were operated at 40 mA and 40 kV by using Cu Kα radiation (λ = 1.54 Å). The scattering angle (2θ) was varied from 5° to 60°, and the step width was 0.04°. The morphology change of samples was observed by a model 1530VP scanning electron microscope (LEO, Oberkochen, Germany). Prior to imaging, the samples were coated with gold in a vacuum. TGA was performed using a Diamond TG-DTA thermogravimetric analyzer (PerkinElmer Co., Waltham, MA, USA). The measurements were conducted under nitrogen flow. The scans were run from 60 to 650 °C at a heating rate of 10 °C/min.

RESULTS AND DISCUSSION

Solubility of Starch Dissolved in [BMIm]Cl. [BMIm]Cl is mostly used to dissolve starch. The Cl^- anions in [BMIm]Cl are able to disrupt the hydrogen bonds among hydroxyl groups

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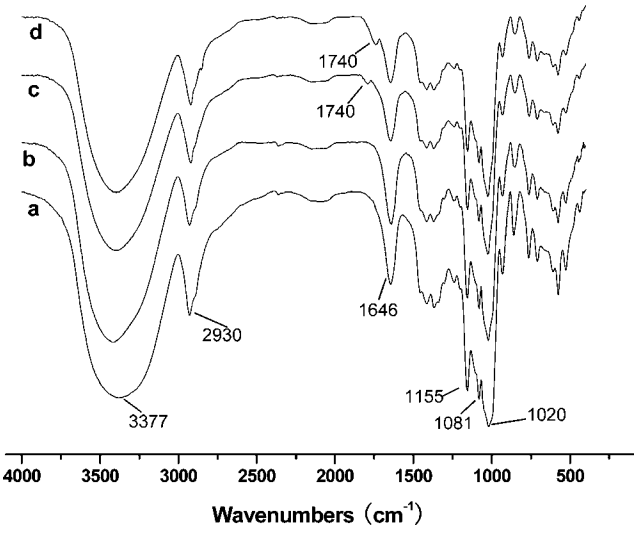

Figure 2. FT-IR spectra: (a) native starch; (b) dried pregelatinized starch; (c) starch laurate (DS 0.102); (d) starch laurate (DS 0.171).

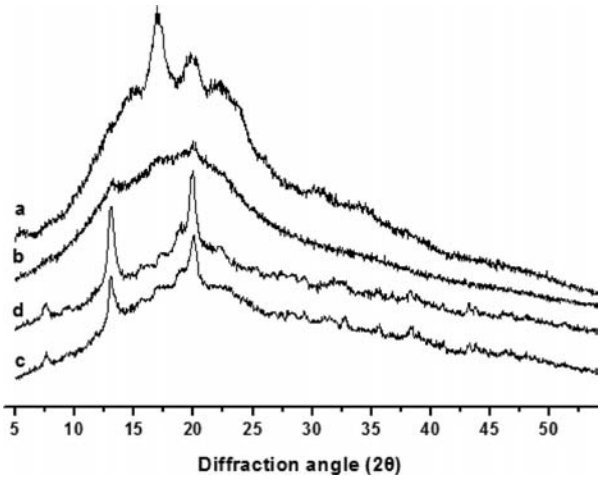

Figure 4. X-ray diffraction spectra: (a) native starch; (b) dried pregelatinized starch; (c) starch laurate (DS 0.102); (d) starch laurate (DS 0.171).

in starch molecules, thus destroying the semicrystalline granular structure of native starch.[14] The assessment of starch dissolution in ionic liquid media was done by visual methods, where the evaluation of starch dissolution is to observe the transparency of starch solution with the observers' own eyes.[26] This visual method may cause more inaccuracy due to the limitation of sensory evaluation. The turbidity of a fluid originates from the suspended particles in the solvent. If the solute is perfectly dissolved, the turbidity would remain constant. When the solution reached saturation, further additions of starch remain insoluble and the turbidity of the solution increases sharply. As a simple technique with high accuracy and reproducibility, turbidimetry was proposed to evaluate the quantitative solubility of starch in solvents.[24] In this experiment, the solubility of high-amylose maize starch dissolved in [BMIm]Cl was evaluated by measuring the turbidity.

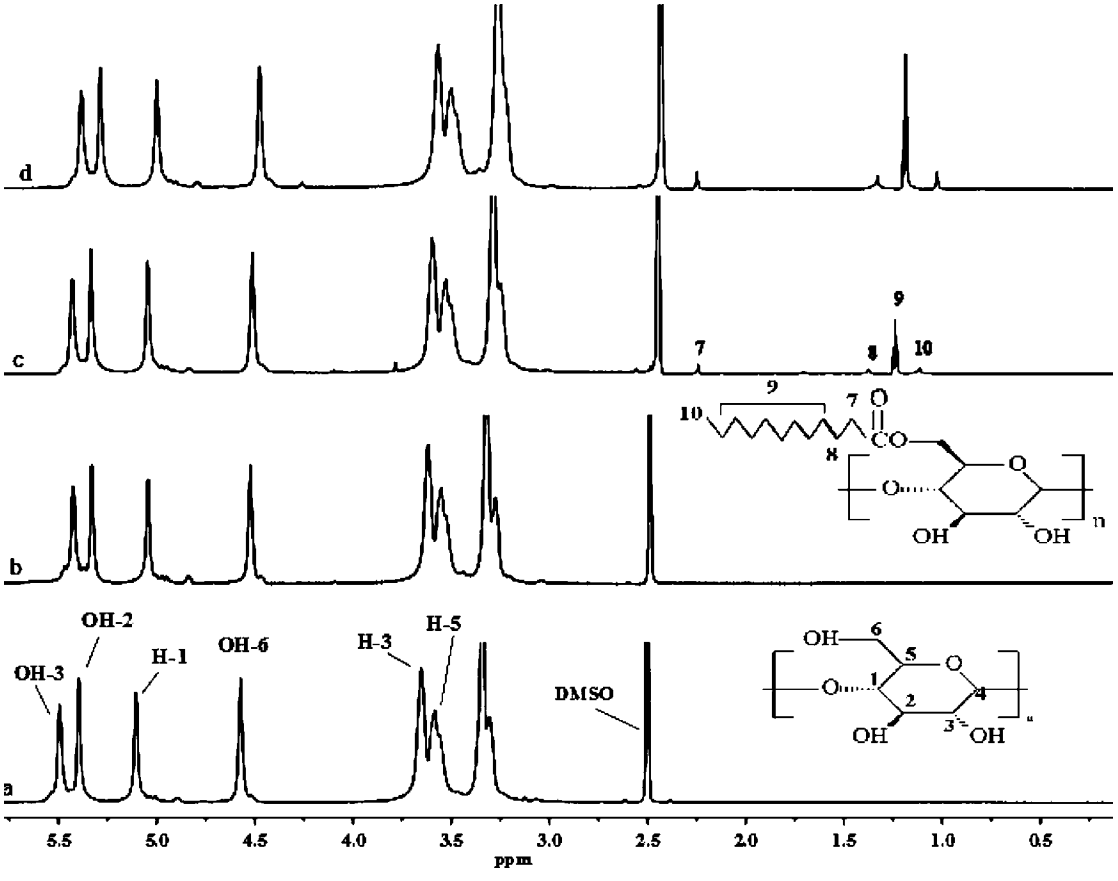

Figure 3. ^{1}H NMR spectra: (a) native starch; (b) dried pregelatinized starch; (c) starch laurate (DS 0.102); (d) starch laurate (DS 0.171).

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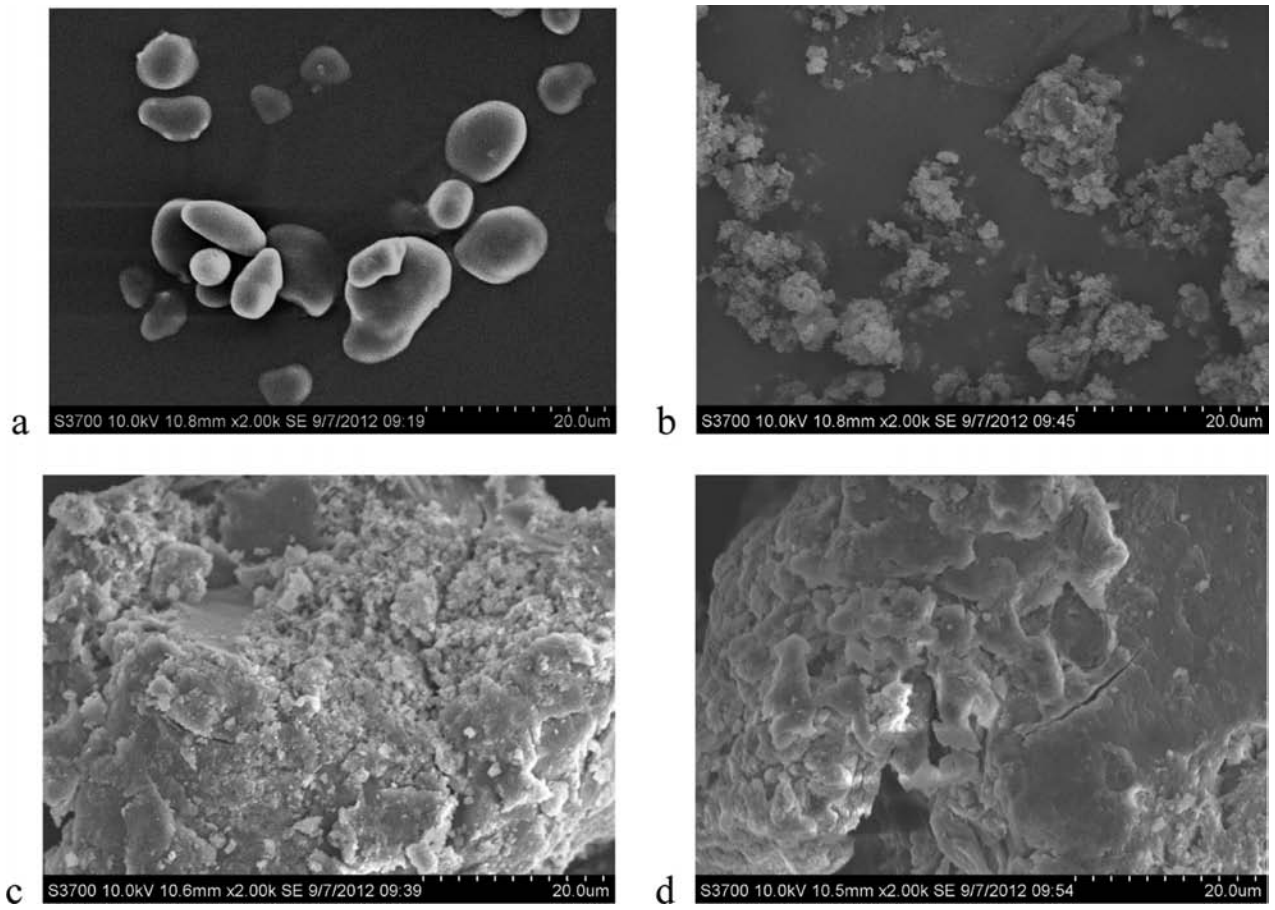

Figure 5. SEM images of starch granules: (a) native starch ×2000; (b) dried pregelatinized starch ×2000; (c) starch laurate ×2000 (DS 0.102); (d) starch laurate ×2000 (DS 0.171).

The turbidity of high-amylose maize starch dissolved in [BMIm]Cl at 100 °C is presented in Figure 1. In the solution process, the turbidity curve of starch dissolved in [BMIm]Cl gave a gradual increasing trend with increasing starch concentration, but the turbidity could be regarded as almost constant as far as the whole scale of turbidity (1−1000 NTU) is concerned. With increasing starch concentration, saturation was reached. Further addition of starch resulted in a sharp increase of turbidity. According to the turbidity experiment, the maximum solubility of high-amylose maize starch was 11.0 g/100 g [BMIm]Cl. On the basis of the maximum solubility of high-amylose maize starch in [BMIm]Cl, high-amylose maize starch is soluble in [BMIm]Cl to around 10% by weight. Therefore, starch was dissolved in [BMIm]Cl at a concentration of 10% to ensure complete gelatinization of starch for the following esterification modification.

Screening of Lipases. Different lipases, including CRL, PPL, lipase PS, lipase TL IM, and Novozym 435, were screened for their catalysis ability to acylate starch with lauric acid in the proposed IL media. The reaction conditions used were as follows: molar ratio of 1:3 (AGU/lauric acid), reaction time of 5 h, and temperature of 60 °C. Lipase dosage was added by 137 U per total weight of substrate. For different lipases, CRL, PPL, lipase PS, lipase TLIM, and Novozym 435, the DS values of starch laurates were 0.165, 0.039, 0.008, 0.083, and 0.013, respectively, which suggested that CRL had the highest incorporation abilities for selected fatty acids. On the basis of the above results, the related catalysis conditions of CRL were further investigated.

Effect of Lipase Dosage. The influence of lipase dosage on the DS value of starch laurate is presented in Table 1. With lipase dosage increased from 0 to 0.3 g, DS of starch laurates rose from 0.004 to 0.165. It is obvious that the efficiency of the esterification process was significantly improved with the addition of lipase, but further increasing the lipase dosage from 0.3 to 0.45 g resulted in only a slight increase in the DS of the product (0.006). The small increase in DS may be due to the fact that the hydroxyl groups have been saturated with laurate groups, creating steric hindrance and reducing the free space for further esterification. Therefore, considering the catalysis effect of the lipase as well as its cost, 0.3 g of lipase was applied in the following research. The maximum DS acquired from previous research on the lipase-catalyzed esterification of fatty acid starch ester in aqueous systems was 0.018,[27] whereas the DS values in this research were much higher than 0.018, which suggested that enzymatic acylation of starch was quite efficient in IL media.

Effect of Molar Ratio of Lauric Acid/AGU. As shown in Table 2, an increase of the molar ratio of lauric acid/AGU from 1 to 3 led to a gradual improvement of the DS value of starch laurates. There was no significant difference in DS when the molar ratio was further enhanced. A probable reason for this phenomenon could also be attributed to the steric hindrance of the existing laurate groups on starch laurate molecules, hindering further esterification reaction. In the present study,

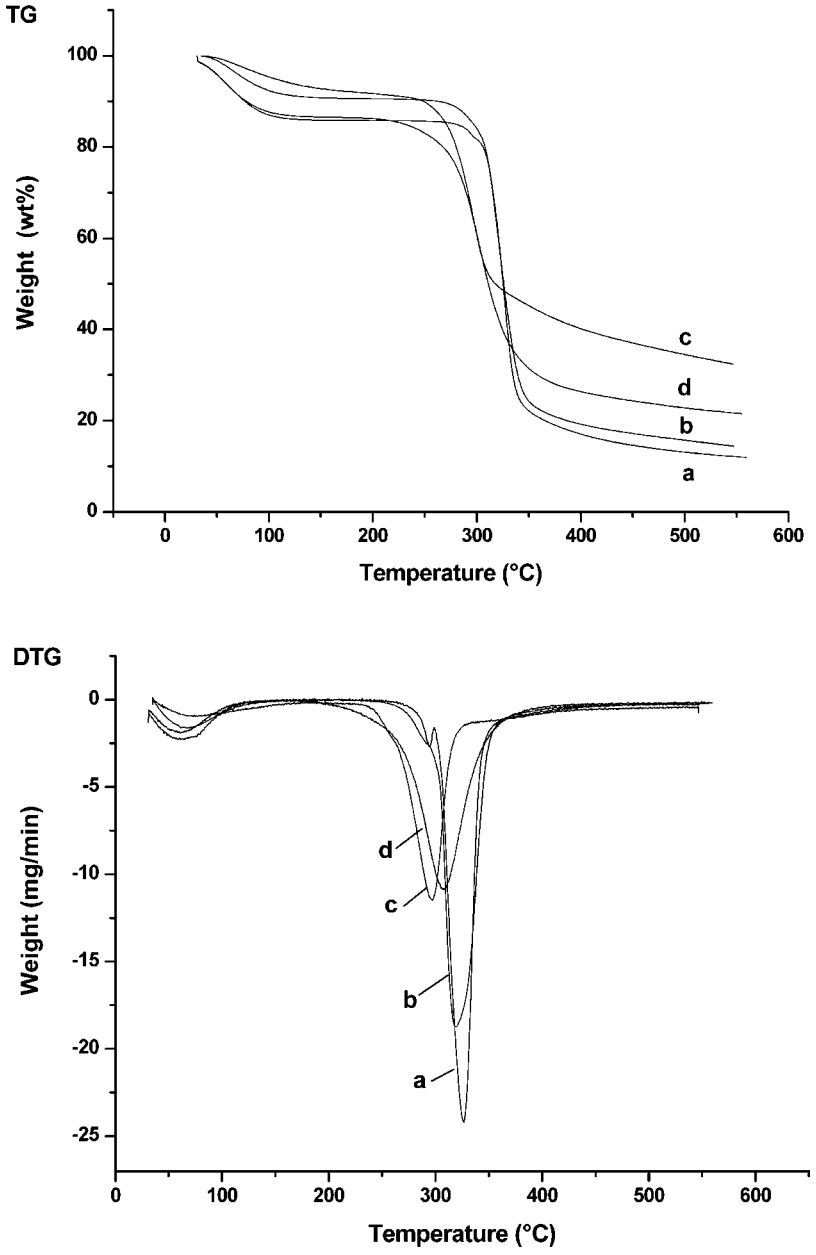

Figure 6. TG and DTG curves: (a) native starch; (b) dried pregelatinized starch; (c) starch laurate (DS 0.102); (d) starch laurate (DS 0.171).

the optimum molar ratio of lauric acid/AGU was 3:1. A similar trend was also reported in a previous paper on the synthesis of fatty acid starch esters in IL medium.[16]

Effect of Reaction Temperature. Different reaction temperatures (50, 60, 70, and 80 °C) were set to investigate the effect of reaction temperature on the esterification process, and the results are presented in Table 3. Reaction temperature plays a vital role in the lipase-catalyzed modification of starch by affecting the penetration speed of reagents as well as the catalysis activity of lipase. When the reaction temperature increased from 50 to 60 °C, the DS of starch laurates was found to rise from 0.048 to 0.165, whereas a further increase in reaction temperature above 60 °C resulted in a sharp decrease in the DS value, which could be probably due to the inactivation of lipase under high temperature. In the earlier research of lipase-catalyzed synthesis of starch palmitates in [BMIm][BF_4]/[BMIm]Ac mixtures, the optimal reaction temperature was also 60 °C,[22] whereas the optimum temperature of CRL under native condition is 37 °C. Accordingly, enzyme temperature stability is usually much higher in ionic liquid media than in aqueous solution.

Effect of Reaction Time. The correlation between the reaction time and DS value is depicted in Table 4. With an increment of reaction time from 3 to 5 h, the DS value of starch laurate rose from 0.102 to 0.165. The DS value dropped gradually when the reaction time was >5 h. The reason may be that the amount of water (from the reagents and as byproduct of the enzymatic esterification reaction) increased with the progress of the reaction, thereby enhancing the water activity of the original system and affecting the pH of the system. As the polarity of solvent influences the hydration level of an enzyme, the high water activity of the system normally leads to low stability of an enzyme.[28,29] Consequently, side reactions (hydrolysis of starch laurate) probably occurred at longer reaction time due to partial deactivation of the enzyme and the increased amount of water molecules in the reaction system. It

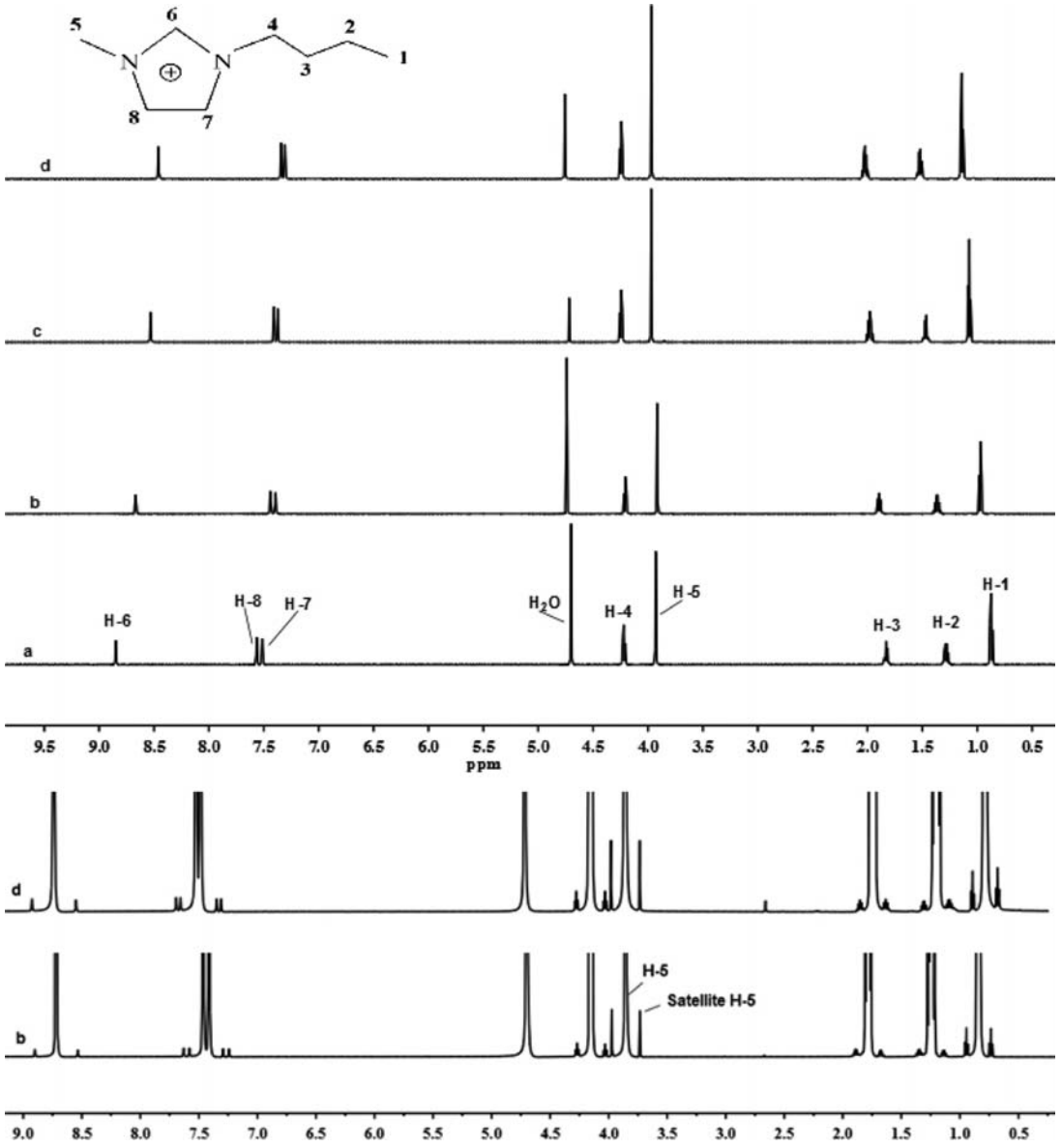

Figure 7. ^{1}H NMR spectra of ILs (top) and expansion of recycled ILs showing the signal of the *N*-methyl ^{13}C satellites (bottom): (a) fresh [BMIm]Cl; (b) recycled [BMIm]Cl; (c) fresh [BMIm][BF_4]; (d) recycled [BMIm][BF_4].

should be noted that the optimal reaction time of lipase-catalyzed synthesis of starch palmitates in [BMIm][BF_4]/[BMIm]Ac mixtures was 3 h,[22] which was shorter as compared to the corresponding value in this work. The difference might be explained by the different chemical modification mechanisms of the two systems. In the [BMIm][BF_4]/[BMIm]Ac homogeneous system, the OH groups of starch molecule exposed to the system make esterification easy to process. However, when pregelatinized starch was esterified in [BMIm]-[BF_4], more time would be needed for the reagent to penetrate into the pregelatinized starch in the heterogeneous system.

FT-IR Analysis. The FT-IR spectra of native starch (Figure 2a), dried pregelatinized starch (Figure 2b), and starch laurate with DS 0.102 (Figure 2c) and DS 0.171 (Figure 2d) are depicted in Figure 2. No obvious difference was observed between spectra of native starch and starch pregelatinized using [BMIm]Cl, where the absorption bands of C−H and O−H stretching vibrations in AGU showed strong signals at 2930 and 3377 cm^{-1}. The bands at 1155, 1081, and 1020 cm^{-1} were attributed to stretching vibrations of C−O bonds.[30] The esterification process and the formation of starch laurate esters were confirmed according to FT-IR analysis. A new band arose at 1740 cm^{-1} in the FT-IR spectra of starch laurates (Figure 2c,d), which originated from C═O of the starch ester group. Besides, the intensity of the ester characteristic peak at 1740 cm^{-1} was enhanced with the increase of DS. Generally, a peak would arise in long-chain fatty acid starch esters at about 2850−2950 cm^{-1}, which originated from the C−H stretching of the aliphatic alkyl chain of acids.[4] However, owing to the relatively low DS of starch ester, only a slight increase was detected at 2930 cm^{-1} in Figure 2d. A similar phenomenon was also observed in previous literature.[31]

^{1}H NMR Spectra Analysis. The ^{1}H NMR spectrum of native starch (Figure 3a), dried pregelatinized starch (Figure 3b), and starch laurate with DS 0.102 (Figure 3c) and DS 0.171 (Figure 3d) are depicted in Figure 3. Native starch and starch pregelatinized using [BMIm]Cl showed no obvious differences in their ^{1}H NMR spectra (Figure 3a,b). The signals at 3.31 ppm

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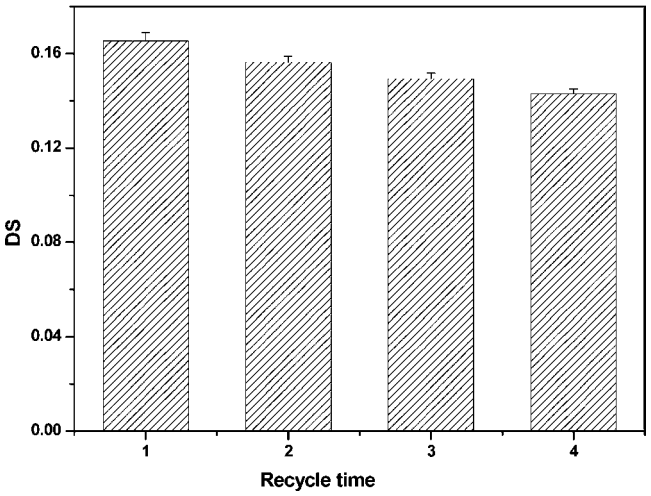

Figure 8. Results of the ionic liquid recycle test. Gelatinization conditions: starch concentration, 10%; gelatinization temperature, 100 °C; gelatinization time, 3 h. Reaction conditions: dried pregelatinized starch, 1.62 g; [BMIm][BF_4], 16.2 g; lipase dosage, 0.3 g; lauric acid/AGU, 3:1; reaction temperature, 60 °C; reaction time, 5 h.

for H-2, 3.64 ppm for H-3, 3.15 ppm for H-4, and 3.57 ppm for H-5 appeared clearly in the spectra. Noticeable peaks distributed between 4.58 and 5.50 ppm were assigned to chemical shifts of H-1 and OH-2,3,6.[32] Compared with native and pregelatinized starch, four new peaks arose in the ^{1}H NMR spectra of starch laureates as hydrogen atoms of hydroxyl groups in starch were substituted by acyl groups during the esterification process. The triplet peak, around 1 ppm, was linked to the three protons in the terminal methyl group of the acyl chain (peak 10 in Figure 3c,d). The proton resonance at 1.4 ppm (peak 8) was associated with the methylene group directly before the carbonyl group, and the peak at 2.25 ppm (peak 7) corresponded to the methylene group beside it. The strong peak at 1.2 ppm (peak 9 in Figure 3c,d) was related to all other methylene groups in the acyl chain.[33] Moreover, the intensities of those four peaks (peaks 7−10) became more pronounced with the increase of DS value in products.

XRD Analysis. X-ray diffraction was conducted to investigate if the crystallinity of starch was changed during the modification process. The XRD patterns of the crystallinity of native corn starch, dried pregelatinized starch, and starch laurate are presented in Figure 4. Native starch exhibited a typical B-type X-ray pattern with strong reflections (2θ) at about 5.5°, 17°, 22°, and 24°.[34] However, only a broad amorphous peak was detected in the X-ray pattern of dried pregelatinized starch. Clearly, the crystal region of native starch was damaged during the gelatinization process, which indicated that the inter- and intramolecular hydrogen bonds in starch molecules were broken by [BMIm]Cl during the gelatinization processes. Similar XRD diagrams were also obtained in a previous study.[13] The X-ray pattern showed that starch laurate (DS 0.171) contained three diffraction peaks (7.8°, 13.5°, and 20.7°), giving a characteristic V-type pattern. The V-type crystallinity of starch laurate, which was different from B-type crystallinity in native starch and amorphous phase in dried pregelatinized starch, could originate from the single-helical structure "inclusion complex" composed of starch molecules and esterified laurate. These results are in accordance with the properties of corn starch laurate found in a previous study.[35]

SEM Analysis. The morphology properties of native starch, dried pregelatinized starch, and starch laurates are illustrated in Figure 5. According to the SEM images, native starch exhibited a mixture of round or oval granules with various sizes. Some granules showed elongated rod shapes. However, granules with irregular triangle shapes were not observed, which was different from results reported for previous research.[36,37] Scanning electron micrographs of starch pregelatinized using [BMIm]Cl showed a complete transformation of original structure of starch granule, which increased its accessibility during the esterification process. For starch laurate, large aggregations or cluster formation was found. The above results indicated that the ordered crystalline structure of native starch granules was damaged in gelatinization and esterification process, which was also demonstrated by X-ray analyses.

Thermal Analysis. The thermogravimetry (TG) and derivative thermogravimetry (DTG) thermograms of native starch, dried pregelatinized starch, and starch laurate are depicated in Figure 6. There was a small weight loss at around 300 °C and a significant loss in the DTG curve of native starch, which were due to the evaporation of water and the decomposition of starch, respectively. The dried pregelatinized starch and starch laurate, however, exhibited only one degradation peak relating to the starch degradation.[38] The maximum decomposition temperatures were found to be 327 °C for native starch, 319 °C for dried pregelatinized starch, 297 °C for starch laurate with DS 0.102, and 310 °C for starch laurate with DS 0.171. Furthermore, the initial degradation temperatures of native starch, dried pregelatinized starch, starch laurate (DS 0.102), and starch laurate (DS 0.171) were found to be about 311, 304, 274, and 282 °C, respectively. These results indicated that lauroylation of starch in the present IL system reduced their initial temperature of thermal degradation as well as their thermal stability compared to that of native starch. These results are in good agreement with the observations in previous literature.[22]

Recycling of the ILs. The pregelatinized starch and starch esters were washed and precipitated with absolute ethanol after modification process. Because ILs have high thermal stability and negligible vapor pressure, they can be recycled easily by filtration and evaporation. The intensity of ^{13}C satellites of the imidazolium *N*-methyl group was used to confirm the purity of recovered ILs by ^{1}H NMR spectroscopy. As the natural abundance of ^{13}C is 1.11%, the intensity of every satellite peak of *N*-methyl singlet is 0.555%.[39] As shown in Figure 7, except for a residual water peak at 4.70 ppm, there were no peaks of other hydrogen-containing compounds existing. Therefore, the purities of recycled [BMIm]Cl and [BMIm][BF_4] were >99%. The recycled ILs were also used to synthesize starch laurate under the same conditions. The results of the recycling experiments are presented in Figure 8. As depicted in the figure, the DS values of starch laurates obtained in the recycled ILs were in good accordance with those obtained in the fresh ILs. As a result, ILs could be recycled and reused because products had high DS in the recycling run.

In this work, an exploratory study of the enzymatic synthesis of starch laurates in ionic liquid systems was investigated using a two-step methodology. Starch laurates with different DS values can be obtained by controlling the reaction conditions. The structures of starch laurates were confirmed by FT-IR and ^{1}H NMR spectroscopy. The semicrystalline structure of native starch was completely destroyed, and new structure was formed in gelatinization and modification processes. The thermal

stability of starch laurates decreased after modification. After reaction, the ionic liquids were effectively recycled and reused. On the basis of the fact that the ILs can be effectively recycled after each modification, this study provides an environmentally friendly procedure for exploring new end-uses of starch.

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Notes

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ABBREVIATIONS USED

[BMIm]Cl, 1-butyl-3-methylimidazolium chloride; [BMIm]-[BF_4], 1-butyl-3-methylimidazolium tetrafluoroborate; AGU, anhydroglucose unit; [BMIm]Ac, 1-butyl-3-methylimidazolium acetic; DMAC, *N*,*N*-dimethylacetamide; DMSO, dimethyl sulfoxide; ILs, ionic liquids; DS, degree of substitution; FT-IR, Fourier transform infrared spectroscopy; XRD, X-ray diffraction; NMR, nuclear magnetic resonance; SEM, scanning electron microscopy; TGA, thermogravimetric analysis; TG, thermogravimetry; DTG, derivative thermogravimetry; NTU, nephelometric turbidity units

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