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# Preparation and Properties of Enzyme-Modified Cassava Starch−Zinc Complexes

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**ABSTRACT:** Starch−zinc complexes were synthesized by reaction of enzyme-modified starch with zinc acetate. The effect of reaction parameters such as hydrolysis rate, reaction temperature, reaction time, pH value, and concentration of zinc acetate on the zinc content and zinc conversion rate was studied. The zinc content and conversion rate of the product prepared under optimal conditions were 100.24 mg/g and 87.06%, respectively. The results of scanning electron microscopy (SEM) demonstrated that the obtained starch−zinc complexes displayed a porous appearance. The results of Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), and $^{13}C$ cross-polarization/magic-angle spinning nuclear magnetic resonance ($^{13}C$ CP/MAS NMR) showed that zinc was mainly coordinated to the oxygen atoms of the glucose unit 6-$CH_2OH$. The formation of starch−zinc complexes was also indirectly confirmed by the results of conductivity measurements. Thermal properties of the complexes were influenced by the zincatation process. This study revealed that nonallergenic starch might be used effectively as a carrier of zinc for zinc supplementation purpose.

**KEYWORDS:** *starch, enzyme-modified starch, zinc, starch−zinc complexes*

## INTRODUCTION

Starch, chemical formula $C_6H_{10}O_5$, is made of a large number of glucose units joined together by glycosidic bonds. As an abundant natural polysaccharide, starch has been chemically modified and widely used in numerous industrial applications.[1] Due to the existence of primary and secondary hydroxyl groups of the constituent glucose unit, starch is prone to many substitution reactions, such as esterification and etherification. Similarly, metal ions can also be coordinated to the hydroxyl oxygen atoms of starch, which leads to the formation of starchates.[2−4]

Considering the potential applications, more and more attention has been paid to the area of metal derivatives of starch in the past decades. For example, bismuth(III) and bismuth(V) derivatives of starch were synthesized as an aid in curing skin diseases and radiocontrasts;[2] aluminum derivatives of starch were suggested to be potentially useful in the treatment of gastric ulcers and dermatological diseases;[5] starch−copper complexes were considered useful for accelerating the healing of topical wounds or as hair growth stimulants;[6] and starch−iron complexes were taken into account as sources of iron in biologically active form.[7]

Zinc is an essential mineral required by the body for maintaining a sense of smell, keeping a healthy immune system, building proteins, triggering enzymes, and creating DNA. Zinc also helps the cells in the body communicate by functioning as a neurotransmitter. A deficiency in zinc can lead to stunted growth, diarrhea, impotence, hair loss, eye and skin lesions, impaired appetite, and depressed immunity.[8] In order to prevent zinc deficiencies, various kinds of zinc derivatives from organic and inorganic zinc compounds have been widely used as zinc fortifying additives. However, many zinc compounds still have the problems of stimulating stomach and low absorption rate. In view of the biological significance of zinc, some attention has been paid to the synthesis of zinc derivatives of starch in recent years. For example, Staroszczyk and Janas[9] synthesized zincatated potato starch in the solid state by microwave irradiation. Woo et al.[10] synthesized zinc-bound starch from cross-linked starch and zinc salt through an extrusion process. However, there are still few reports about synthesizing starch−zinc complexes in aqueous solution.

Previous studies[11,12] have shown that starch granule microstructural features may play a role in reagent accessibility. Whistler et al.[13] observed that corn starch pretreated with glucoamylase prior to esterification showed esterification levels 5 times greater than native corn starch. Their dye experiment showed derivatization not only on the surface but also in the granule central interior, which indicated reagent penetration to the granule interior through pores and derivatization of a lower density porous center. Karim et al.[14] found that partial enzyme hydrolysis of starch in the granular state enhanced the subsequent hydroxypropylation and significantly increased the molar substitution. In a previous study,[15] we had reported that enzymatic pretreatment could improve the reaction processes significantly and increase the degree of substitution (DS) of amphoteric starch from enzyme-modified starch under the same reaction conditions. Metal derivatives of starch were synthesized with native starch as raw material in a previous study. In this paper, a new method was proposed to coordinate zinc to starch by reaction of zinc acetate with cassava starch, which was hydrolyzed by a mixture of $\alpha$-amylase and glucoamylase in aqueous solution. The effect of hydrolysis

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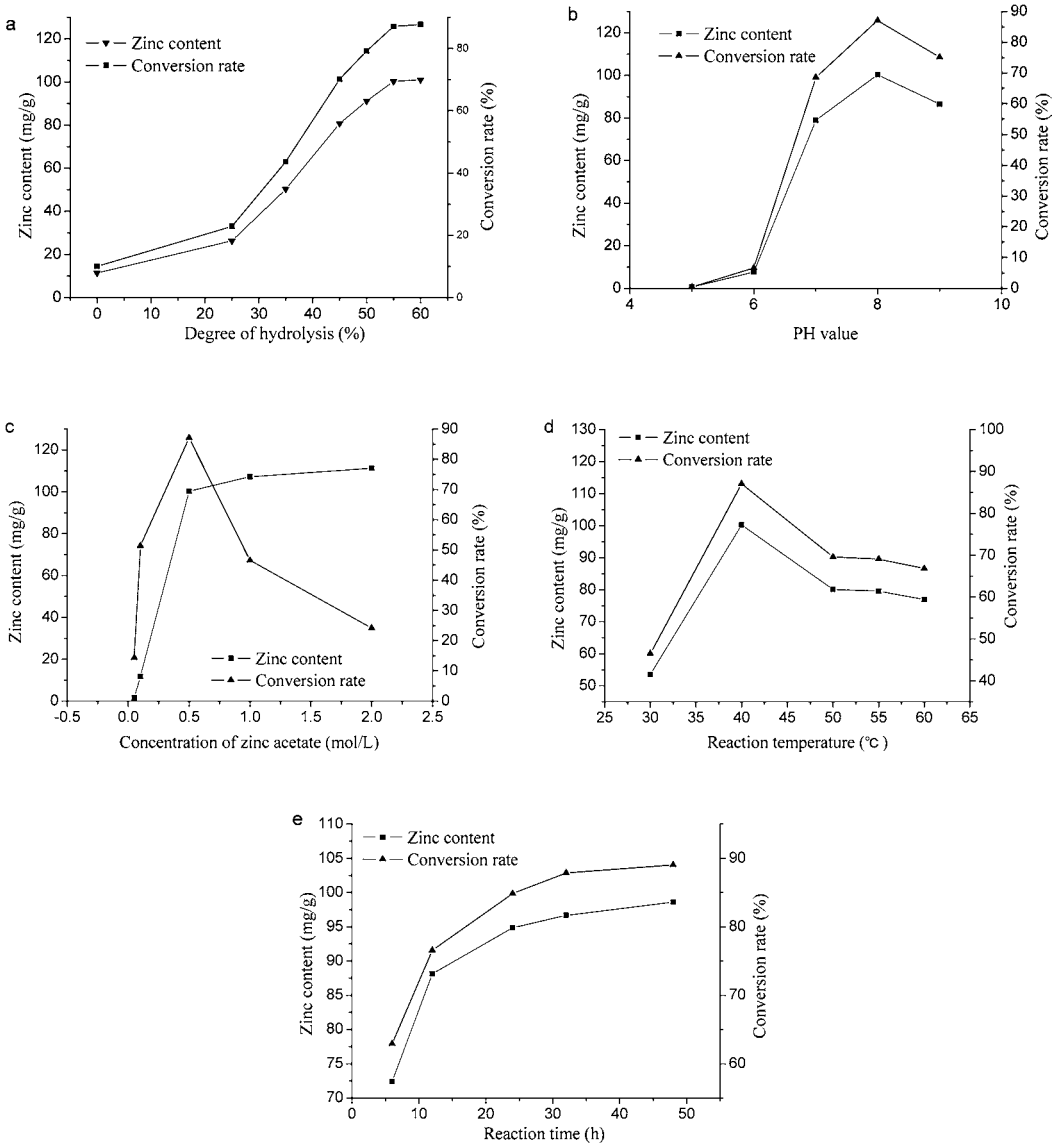


**Figure 1.** Effect of reaction parameters on zinc content and conversion rate. (a) Effect of hydrolysis rate (reaction temperature 40 °C, pH 8.0, reaction time 24 h, concentration of zinc acetate solution 0.5 mol/L). (b) Effect of pH value (hydrolysis rate 55.3%, reaction temperature 40 °C, reaction time 24 h, concentration of zinc acetate solution 0.5 mol/L). (c) Effect of concentration of zinc acetate (hydrolysis rate 55.3%, reaction temperature 40 °C, pH 8.0, reaction time 24 h). (d) Effect of reaction temperature (hydrolysis rate 55.3%, pH 8.0, reaction time 24 h, concentration of zinc acetate solution 0.5 mol/L). (e) Effect of reaction time (hydrolysis rate 55.3%, pH 8.0, reaction temperature 40 °C, concentration of zinc acetate solution 0.5 mol/L).

rate, reaction temperature, reaction time, pH value, and concentration of zinc acetate on the zinc content and conversion rate was investigated. In addition, the obtained starch−zinc complexes were characterized by scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FT-IR), $^{13}C$ cross-polarization/magic-angle spinning nuclear magnetic resonance ($^{13}C$ CP/MAS NMR), and differential scanning calorimetry (DSC).

## MATERIALS AND METHODS

**Materials.** Commercial food-grade cassava starch (QR-CS12) was obtained from Foshan Qirun Starch Co. Ltd. (Foshan, China), which was produced according to the industrial standard NY/T 875-2012 in China. Both α-amylase (E.C. 3.2.1.1., activity 4000 units/g) and glucoamylase (E.C. 3.2.1.3., activity 100 000 units/g) were supplied by Xuemei Enzyme Technology Co. (Wuxi, China). One unit of α-amylase activity corresponds to the amount of enzyme that liberates 1 g of soluble starch per hour at pH 6.0 at 60 °C. One unit of glucoamylase activity is defined as the amount of enzyme that liberates 1 mg of glucose from soluble starch per hour at pH 4.6 at 40 °C. Zinc acetate was purchased from Tianjin Fuchen Chemical Reagent Co. (Tianjin, China). All of the chemicals used were of analytical grade.

**Preparation of Enzyme-Modified Starch.** Starch (50 g, dry basis) was mixed with 200 mL of acetic acid buffer (pH 5.5) in a 250 mL conical flask and preheated in a thermostated water bath at 55 °C with stirring for 20 min. α-Amylase (100 mL) and glucoamylase solution (125 mg of α-amylase and 250 mg of glucoamylase dissolved in 100 mL of pH 5.5 acetic acid buffer) were added, and then the mixture was incubated at 55 °C for 12 h. Samples were filtered, washed in water, dried in a convection oven at 45 °C overnight, and then ground with a mortar and pestle. The hydrolysis percent reducing value of all samples was determined by the method of Bruner.[16] Hydrolysis rate of the sample made under the above conditions was 55.3%. Enzyme-modified samples with different hydrolysis rates were obtained by controlling the reaction time.


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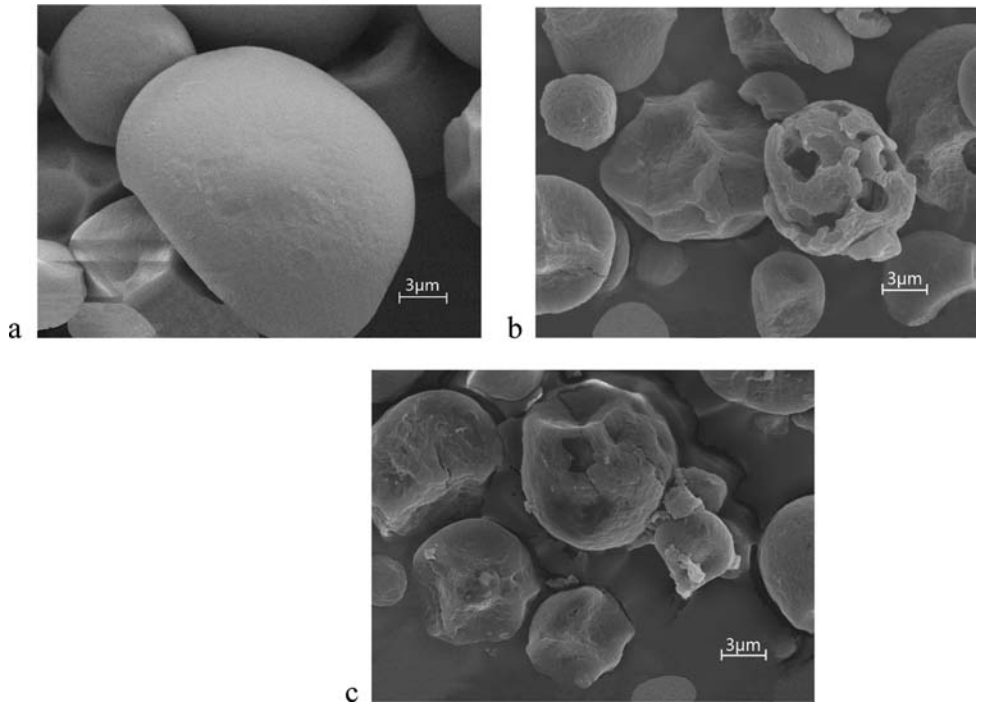


**Figure 2.** Scanning electron micrographs (SEM, ×4000) of (a) native starch, (b) enzyme-modified starch, and (c) starch−zinc complexes.

**Preparation of Starch−Zinc Complexes.** Enzyme-modified starch (50 g, dry basis) with different hydrolysis rates (25.6%, 35.1%, 45.5%, 50.2%, 55.3%, and 60.2%) was dispersed in zinc acetate solution of different concentrations (0.05, 0.5, 1, or 2 mol/L) by a mechanical stirrer. The pH of the mixture was adjusted to a certain value (5, 6, 7, 8, or 9) by adding 0.5 mol/L NaOH or 0.5 mol/L HCl solution. After reaction in a thermostated water bath at a certain temperature (30, 40, 50, 55, and 60 °C for 6, 12, 24, 32, and 48 h, respectively), the mixture was neutralized with 0.5 mol/L HCl to pH 4.5, filtered, washed by deionized water to remove all residual free zinc ions, and dried in an oven at 40 °C overnight. The products prepared under optimal conditions were used for further analysis.

**Determination of Zinc Content and Zinc Conversion Rate.** The zinc content in the samples, after their wet mineralization with $HNO_3$ and $HClO_4$, was determined on a Z-5000 flame atomic adsorption spectrophotometer (AAS) (Hitachi co. Ltd., Tokyo, Japan) according to a standard procedure.[17] The zinc conversion rate ($Y$) was calculated as

$$Y = \frac{(m_1 - m_0)}{m} \times 100 \quad (1)$$

where $m_0$ (grams) is the zinc content of the enzyme-modified starch, $m_1$ (grans) is the zinc content of the starch−zinc complexes, and $m$ (grams) is the zinc content of the zinc acetate added.

**Characterization of Starch−Zinc Complexes.** 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 double-sided sticky tape, followed by coating with gold under vacuum before examination. FT-IR spectra of samples were acquired on a Nicolet 510 spectrophotometer (Thermo Electron Corp., Waltham, MA) by the KBr disk technique. The samples were mixed with anhydrous KBr and then compressed into thin disk-shaped pellets. The spectra were obtained with a resolution of 2 $cm^{-1}$ in a wavenumber range of 400−4000 $cm^{-1}$. An Axis Ultra DLD spectrometer (Kratos Analytical Ltd., Manchester, U.K.) equipped with an Mg K$\alpha$ X-ray source operated at 300 W was employed to investigate the elemental and chemical composition of the sample surfaces. A pass energy of 29.350 eV was used for narrow scans. Samples were scattered on conductive adhesive tape. All binding energies of the peaks were normalized to the position of the main component of the C 1s band at 284.6 eV. $^{13}C$ CP/MAS NMR experiments were performed on a Bruker Avance 400 MHz spectrometer (Bruker Corp., Fallanden, Switzerland) operating at 100 MHz by using a standard broadband MAS probe for 4 mm rotors. Samples (ca. 100 mg) were packed in a 4 mm zirconia rotor and spun at 5 kHz. The spectra were obtained at 20 °C with a 90° pulse width of 4.85 $\mu$s, a contact time of 1 ms, and a relaxation delay time of 2 s. A total of 5000 scans were accumulated for each spectrum. All chemical shifts were reported in parts per million (ppm). Conductivity measurements were determined at room temperature (25 °C) according to previous literature[18] with little change. The conductivity values of zinc acetate solution (0.5 mol/L), aqueous suspensions of enzyme-modified starch and starch−zinc complexes [1.0 g (dry basis) of starch in 20 $cm^3$ of water] and aqueous blends of pasted enzyme-modified starch with zinc acetate [1.0 g (dry basis) of starch in 20 $cm^3$ of 0.5 mol/L aqueous zinc acetate solution] were measured on a DDS-307A conductometer (Shanghai Precision Scientific Instrument Co. Ltd., Shanghai, China) after the samples were heated in a boiling water bath with stirring for 30 min and cooled to room temperature. Results were stable within 24 h. All measurements were replicated three times. Gelatinization temperatures were measured and recorded on a Perkin-Elmer DSC-8000 (PerkinElmer Co., Waltham, MA) differential scanning calorimeter, equipped with a thermal analysis data station. Water (7 $\mu$L) was added with a microsyringe to starch (3 mg, dry basis) in the DSC pans, which were then sealed, reweighed, and kept overnight at room temperature. The scanning temperature range was 30−120 °C and the heating rate was 10 °C/min. Thermograms were recorded with an empty pan as a reference.

## RESULTS AND DISCUSSION

The coordination of zinc to enzyme-modified starch is obviously a complex reaction system influenced by many factors. The effect of reaction parameters (hydrolysis rate, pH value, concentration of zinc acetate, reaction temperature, and reaction time) on the zinc content and conversion rate was studied, and the results are shown in Figure 1.

**Effect of Hydrolysis Rate.** As shown in Figure 1a, when native starch was used, the zinc content and conversion rate


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were very low. However, zinc content and conversion rate gradually increased at the first stage with the degree of hydrolysis rate increasing. A mixed $\alpha$-amylase and glucoamylase system hydrolyzes starch granules efficiently, which makes the surface of starch granules rough and increases the surface area. The increase in surface area can provide more hydroxyl groups to react with reagents. Moreover, the pores or cracks on the surface (Figure 2) caused reagents such as zinc ions to infiltrate into the inner parts of the starch easily, which also caused more reagents to react with hydroxyl groups of enzyme-modified starch granules. The same results were also found by Gao et al.,[15] in which enzymatic pretreatment increased the degree of substitution of the amphoteric starch significantly. Hydrolysis rate of 55.3% was found to reach maximum zinc content and conversion rate. When the hydrolysis rate increased beyond 55.3%, enzyme-modified starch did not have good porous structure, which was unfavorable for the reaction between starch and zinc salt because of excessive hydrolysis.

**Effect of pH Value.** As presented in Figure 1b, the pH value had a very important effect on zinc content and conversion rate. When the pH value increased from 5 to 8, the zinc content and conversion rate increased rapidly from 0.57 to 100.24 mg/g and from 0.43% to 87.06%, respectively. The reason was probably that the increase in pH value favored the deprotonation of hydroxyl groups. This result was in accordance with that of Nagy and Szorcsik,[19] who reported that the -OH groups of carbohydrates could form much stronger complexes with cations in alkaline solution than in neutral solution. However, further increase in pH value above 8 reduced the zinc content and conversion rate. From the above analysis, we could draw the conclusion that the optimal pH value was 8.

**Effect of Concentration of Zinc Acetate.** The influence of zinc acetate concentration on the zinc content and conversion rate was investigated and the result is shown in Figure 1c. Zinc content and conversion rate increased with increasing zinc acetate concentration at the first stage. However, when the concentration was beyond 0.5 mol/L, the zinc content remained almost constant with increasing concentration of zinc acetate. Hence, the conversion rate was decreased sharply according to eq 1. Thus, the optimal concentration of 0.5 mol/L was selected for the following runs.

**Effect of Reaction Temperature.** As presented in Figure 1d, when the reaction temperature was below 40 °C, zinc content and conversion rate increased with increasing temperature. However, further increase in reaction temperature above 40 °C reduced the zinc content and conversion rate. A plausible reason is that high temperature damaged easily the porous structure of enzyme-modified starch during a long period of reaction, which favored the formation of starch−zinc complexes. Similar results were found by Tomasik et al.,[7] who observed that temperature elevation did not accelerate the reaction and even resulted in inhibition.

**Effect of Reaction Time.** The influence of reaction time on the zinc content and conversion rate is shown in Figure 1e. As the reaction time increased, the zinc content and conversion rate showed a trend of increase. At the starting hours, zinc content and conversion rate increased very rapidly. When the reaction time was beyond 24 h, the change tended to be steady. When the reaction efficiency and cost are considered, the appropriate reaction time was 24 h.

**Scanning Electron Microscopic Analysis.** SEM was used to investigate the morphology of native starch, enzyme-modified starch, and starch−zinc complexes. The surfaces of native cassava starch granules are smooth (Figure 2a). As shown in Figure 2b, after hydrolysis by $\alpha$-amylase and glucoamylase, the enzyme-modified starch granules displayed a rough and eroded appearance; small holes were observed at the surface and some of the small holes were extended to the interior of the granules. The same results were found by Chen et al.,[20] who indicated that the surface area and adsorption capacity of enzyme-modified starch were significantly improved. After the reaction (Figure 2c), the surface of the starch granules became rougher and a partially deformed morphology with some leached materials was observed. This was probably due to reaction of starch with zinc acetate during the preparation of starch−zinc complexes.

**Fourier Transform Infrared Analysis.** Figure 3 illustrates the FT-IR spectra of zinc acetate, native starch, enzyme-modified starch, and starch−zinc complexes. As shown in Figure 3a, the spectrum of zinc acetate was characterized by bands at 1571 and 1447 $cm^{-1}$, indicating symmetric and antisymmetric stretching vibrations of the carboxyl groups.[21] In the spectrum of native starch (Figure 3b), the extremely broad band at 3377 $cm^{-1}$ and the peak at 2931 $cm^{-1}$ correspond to O−H and C−H stretching, respectively. Three characteristic peaks at 1157, 1081, and 1018 $cm^{-1}$ were attributed to the C−O stretching vibrations,[22] and the peak at 1643 $cm^{-1}$ was assigned to the $\delta$(OH) bending vibration.[23] As can be seen from Figure 3c, the spectrum of enzyme-modified starch was almost the same as that of native starch, indicating that the functional groups of enzyme-modified starch were not changed after the process of enzymatic hydrolysis.

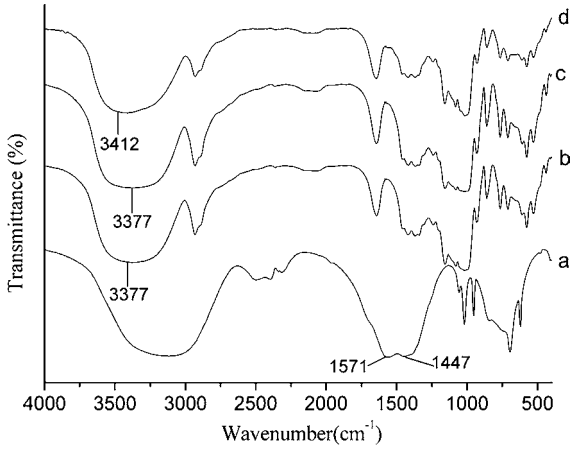


**Figure 3.** FT-IR spectra of (a) zinc acetate, (b) native starch, (c) enzyme-modified starch, and (d) starch−zinc complexes.

As shown in Figure 3d, after reaction with zinc acetate, most of the adsorption peaks of starch−zinc complexes were similar to that of enzyme-modified starch, suggesting that they had the same basic skeleton. Moreover, the peaks attributed to the carboxyl groups were not observed in Figure 3d, indicating that acetate anions were not involved in the starch−zinc complexes. However, the broad band at 3377 $cm^{-1}$ turned narrower, which indicated a reduction of the hydrogen bonds in zincatated starch. Hydroxyl groups of the glucose units are involved in hydrogen bonds, so the reduction of hydrogen bonds suggested that the hydroxyl groups might be involved in coordination with zinc. Similar results were found by Staroszczyk and Janas.[9] Meanwhile, this peak was significantly shifted toward a higher

wavenumber, which was probably a result of the zincatation of hydroxyl groups.

**X-ray Photoelectron Spectroscopic Analysis.** The XPS spectra of enzyme-modified starch and starch−zinc complexes taken in a routine manner are shown in Figures 4a and 5a. Also

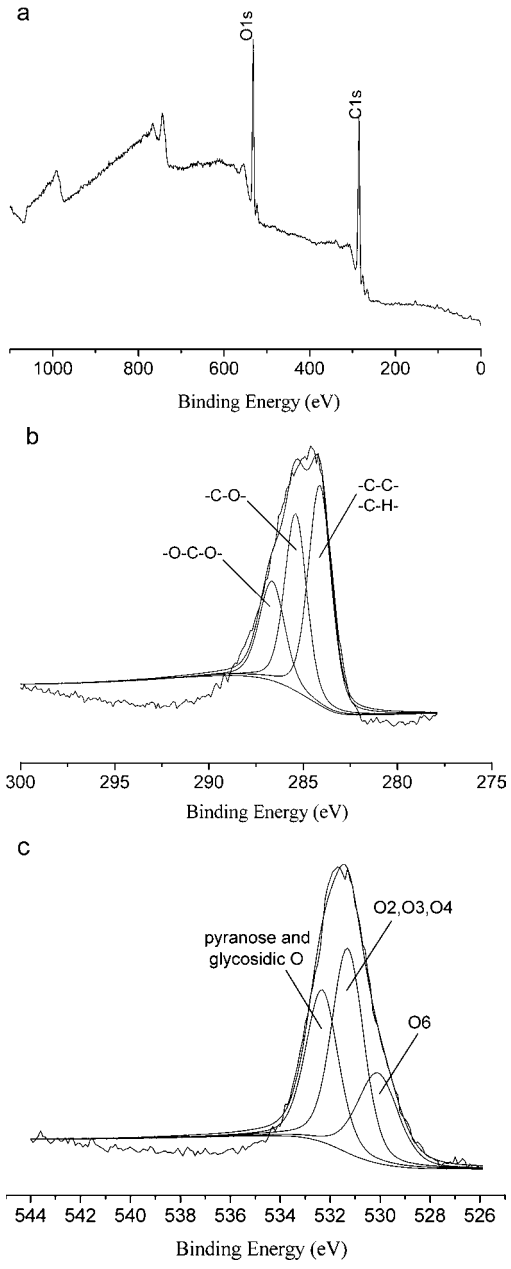


**Figure 4.** XPS spectra of enzyme-modified starch. (a) Routine X-ray photoelectron spectrum; (b) Gaussian analysis of C 1s peaks; (c) Gaussian analysis of O 1s peaks.

shown are spectra in the regions of binding energies of carbon (275−300 eV; Figures 4b and 5b), oxygen (525−545 eV; Figures 4c and 5c), and zinc element (1015−1060 eV; Figure 5d) after expansion and Gaussian analysis. The relevant data for enzyme-modified starch and starch−zinc complexes are collected in Table 1.

The three components hidden within one intensive peak in the carbon region were situated at 284.12, 285.40, and 286.66 eV, which were contributions from -C−C-/-C−H-, -C−O-, and -O−C−O- bonds, respectively, according to the results studied by Sreedhar et al.[24] The three components hidden within one intensive peak in the oxygen region were located at 530.10, 531.30, and 532.33 eV; these peaks belong respectively to the O6; O2, O3, and O4; and pyranose and glycosidic bond O atoms. Assignment of the O 1s peaks was based on the previous literature.[7,19] As can be seen from Figure 4 and Table 1, the main surface components of enzyme-modified starch were carbon and oxygen, and Gaussian analysis of both the C 1s and O 1s bands gave three components.

As presented in Figure 5 and Table 1, two of the components in starch−zinc complexes (284.21 and 285.51 eV) were similar to those of the enzyme-modified starch (284.12 and 285.40 eV), while the binding energy of the remaining component (287.05 eV) was increased by 0.39 eV compared with the 286.66 eV of enzyme-modified starch. Meanwhile, all of the full widths at half-maximum (fwhm), areas, and area proportions of C 1s peaks were changed. This indicated that the electronic situation of carbon atoms was affected by the reaction between enzyme-modified starch and zinc salt.

The oxygen atoms of starch molecules are directly involved in the interactions with metal salts by the valence bond.[7,25] In this study, the coordination of zinc to enzyme-modified starch was also clearly reflected by the remarkable changes of the O 1s peak of starch−zinc complexes. Besides the three components observed in the spectrum of enzyme-modified starch, a new component appeared at 533.60 eV. According to the previous literature,[7] the new component should be a result of the formation of $O^{+}-Zn^{-}$ coordination bond. This fact was also revealed by a shift of the Zn 2p peaks of zinc. The binding energies of Zn 2p of starch−zinc complexes were located at 1020.75 and 1043.85 eV, which was lower than that of zinc element (1021.45 and 1044.55 eV) excited by Mg K$\alpha$.[26] The shifts of the binding energies of oxygen and zinc were probably due to the transference of lone electron pairs from starch molecules to zinc. As a result, the negative charges of zinc increased and the binding energy decreased. On the contrary, the binding energy of corresponding oxygen atoms of starch−zinc complexes increased. Moreover, the area of the component located at 530.76 eV significantly decreased after the reaction, while the other two components located at 531.75 and 532.62 eV changed little. According to the study by Tomasik et al.,[25] the lowest binding energy of the oxygen region corresponded to the O6 atoms of the glucose unit. In this study, considering the decrease of the area of the component located at the lowest binding energy and the emergence of the new component, we drew the conclusion that zinc ions were mainly coordinated to the 6-$CH_2OH$ groups. After the coordination, the binding energy (530.76 eV) of part of the O6 atoms shifted to higher energy (533.60 eV) and the peak area at 530.76 eV decreased significantly. A possible reason for the involvement of O6 in the reaction with zinc was that the electron density of the oxygen atoms of C6 hydroxyl groups is the highest, which is the easiest to react with metal salts.[2]

From the above analysis, we could summarize that zinc ions were mainly presented in the 6-$CH_2OZn$ form and other oxygen atoms of the glucose unit might also participate in the reaction in the starch−zinc complexes because the positions and areas of the other oxygen atoms were also slightly changed. This result was in agreement with the results between starch and thallium,[27] iron,[7] and titanium,[28] in which the O 1s peak experienced changes around 530−535 eV of the resulting starch−metal complexes.


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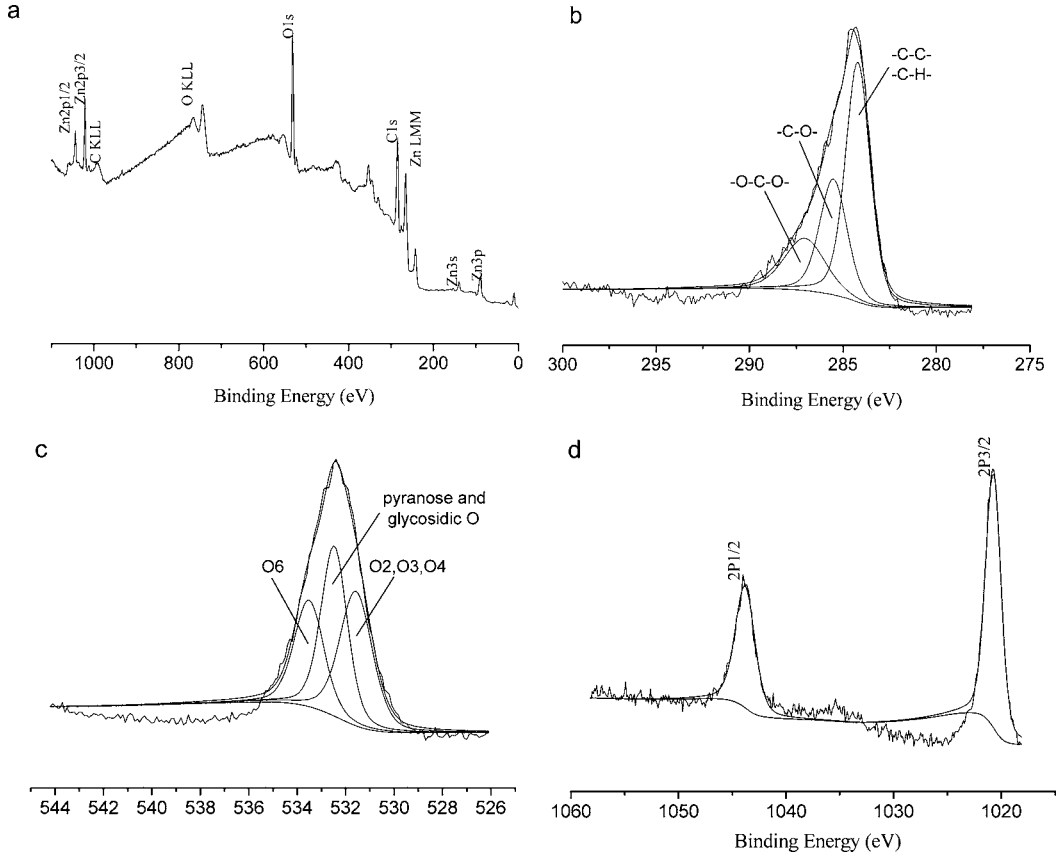


**Figure 5.** XPS spectra of starch−zinc complexes. (a) Routine X-ray photoelectron spectrum; (b) Gaussian analysis of C 1s peaks; (c) Gaussian analysis of O 1s peaks; (d) Gaussian analysis of Zn 2p peaks.

**Table 1. Characteristics of X-ray Photoelectron Spectra of Enzyme-Modified Starch and Starch−Zinc Complexes**

| C 1s | | | | O 1s | | | |
|---|---|---|---|---|---|---|---|
| position (eV) | fwhm | area | area proportion (%) | position (eV) | fwhm | area | area proportion (%) |
| Enzyme-Modified Starch | | | | | | | |
| 284.12 | 1.44 | 13 206.37 | 44.09 | 530.10 | 1.93 | 9746.55 | 24.37 |
| 285.40 | 1.37 | 10 155.17 | 33.91 | 531.30 | 1.48 | 17 173.97 | 42.94 |
| 286.66 | 1.59 | 6589.94 | 22.00 | 532.33 | 1.54 | 13 078.01 | 32.70 |
| Starch−Zinc Complexes | | | | | | | |
| 284.21 | 1.56 | 16 991.44 | 52.67 | 530.76 | 1.11 | 2196.27 | 7.97 |
| 285.51 | 1.73 | 9134.20 | 28.31 | 531.75 | 1.15 | 9104.86 | 33.05 |
| 287.05 | 2.58 | 6136.32 | 19.02 | 532.62 | 1.15 | 9689.63 | 35.17 |
| | | | | 533.60 | 1.38 | 6559.34 | 23.81 |

**$^{13}$C Cross-Polarization/Magic-Angle Spinning NMR Analysis.** The $^{13}$C NMR spectra of enzyme-modified starch and starch−zinc complexes are presented in Figure 6. In the spectrum of enzyme-modified starch, the peaks were assigned as follows: 61.70 ppm, C6; 71.82 ppm, C2, C3, and C5; 81.73 ppm, C4; and 100.90 ppm, C1.[29] In comparison with enzyme-modified starch, the $^{13}$C chemical shifts of C-6 of starch−zinc complexes moved 0.96 ppm toward low field, which indicated that the chemical environment of C6 changed and the shielding effect was enhanced. However, the chemical shifts of the other three peaks changed little. From the combined results of with FT-IR and XPS spectroscopy, we could conclude that zinc salt reacted with enzyme-modified starch mainly on the hydroxyl group of C6. Moreover, the adsorption peak of acetate (around 150−220 ppm) did not appear in the spectrum of starch−zinc complexes. This suggested that zinc acetate was not involved in starch−zinc complexes, in accordance with the FT-IR results.

**Conductivity Measurement Analysis.** Conductivity measurement is a method that was widely used in studying the formation of starch−metal complexes, and the effect of coordination upon the changes in conductivity is an important property that strongly distinguished between the metal complexes of starch and starch polysaccharides.[18,30] In this study, the conductivity values of both zinc acetate solution and aqueous blends of enzyme-modified starch and zinc acetate exceeded the full scale range of the conductivity meter. The conductivity values of enzyme-modified starch and starch−zinc complexes are shown in Table 2. As can be seen from Table 2, the conductivity value of enzyme-modified starch was very low. After the coordination of zinc to enzyme-modified starch, the conductivity value of starch−zinc complexes was higher, but it


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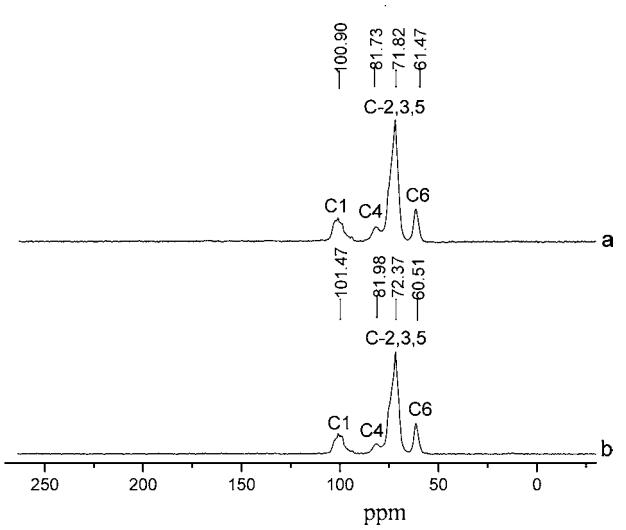


**Figure 6.** $^{13}C$ CP/MAS NMR spectra of (a) enzyme-modified starch and (b) starch−zinc complexes.

was much lower than the values (>10 mS/cm) of physical blends of enzyme-modified starch and zinc acetate (which exceeded the full scale). The results were in line with the report by Ciesielski and Tomasik,[30] who studied the conductivity of the complexes of starch with selected metals and found that the conductivity was significantly decreased compared with the salt solutions after the formation of starch−metal complexes.

**Differential Scanning Calorimetric Analysis.** Gelatinization characteristics of enzyme-modified starch and starch−zinc complexes are shown in Table 2. As can be seen from Table 2, the thermal properties of starch were influenced by the zincatation process. Compared with enzyme-modified starch, the onset ($T_o$), midpoint ($T_p$), and conclusion ($T_c$) temperatures of starch−zinc complexes were shifted to higher temperature. The increase in $T_o$ was in agreement with the proposed melting of the weakest crystallites, and that in $T_c$ reflected the formation of crystallites with a higher melting point (higher crystal perfection or crystals with less water) than those present in the enzyme-modified starch. The $\Delta H$ value primarily reflect the loss of double-helical order.[31] The decrease in $\Delta H$ value of starch−zinc complexes suggested that some of the double helices present in the crystalline and noncrystalline regions were disrupted during the reaction process.

In conclusion, starch−zinc complexes were synthesized by reaction of zinc acetate and cassava starch hydrolyzed by a mixture of $\alpha$-amylase and glucoamylase, which is a facile method to obtain zinc supplementation. The optimum conditions for preparing the starch−zinc complexes were concluded as follows: hydrolysis rate 55.3%, pH 8.0, reaction temperature 40 °C, reaction time 24 h, and zinc acetate concentration 0.5 mol/L. The zinc content and conversion rate observed under optimum conditions were 100.24 mg/g and 87.06%, respectively. The enzyme-modified starch displayed a porous appearance on the surface. After reaction with zinc acetate, the surface of the starch granules became rougher and a partially deformed morphology with some leached materials was observed. In the starch−zinc complexes, zinc ions were mainly coordinated to the oxygen atoms of the glucose unit 6-$CH_2OH$; other hydroxyl groups might also be involved in the reaction. Compared with enzyme-modified starch, starch−zinc complexes had higher $T_o$, $T_p$, and $T_c$ and lower $\Delta H$ values.

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### Notes

The authors declare no competing financial interest.

## ABBREVIATIONS USED

DS, degree of substitution; FT-IR, Fourier transform infrared spectroscopy; $^{13}C$ CP/MAS NMR, $^{13}C$ cross-polarization/magic-angle spinning nuclear magnetic resonance; SEM, scanning electron microscopy; XPS, X-ray photoelectron spectroscopy; DSC, differential scanning calorimetry; AAS, atomic adsorption spectrophotometer

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**Table 2. Differential Scanning Calorimetry Characteristics and Conductivity of Enzyme-Modified Starch and Starch−Zinc Complexes**[a]

| | DSC characteristics | | | | |
|---|---|---|---|---|---|
| sample | $T_o$ (°C) | $T_p$ (°C) | $T_c$ (°C) | $\Delta H$ (J/g) | conductivity (mS/cm) |
| enzyme-modified starch | 60.85 ± 0.37 b | 65.21 ± 0.41 b | 71.97 ± 0.19 b | 10.98 ± 1.09 a | 0.196 ± 0.005 b |
| starch−zinc complexes | 65.13 ± 0.29 a | 70.51 ± 0.53 a | 76.83 ± 0.32 a | 6.86 ± 0.87 b | 0.416 ± 0.018 a |

[a]Data represent mean ± standard deviation. Values in the same column with different letters are significantly different ($p < 0.05$).

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