



Study on the morphology, crystalline structure and thermal properties of yam starch acetates with different degrees of substitution

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This study was carried out to understand and establish the changes in physicochemical properties of starch extracted from Chinese yam (*Dioscorea opposita* Thunb.) after acetylation. Yam starch acetates with different degrees of substitution (DS) were prepared by the reaction of yam starch with glacial acetic acid/acetic anhydride using sulfuric acid as the catalyst. Their formation was confirmed by the presence of the carbonyl signal around 1750 cm⁻¹ in the Fourier transform infrared (FT-IR) spectra. The thermal behavior of the native starch and starch acetate were investigated using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The results reveal that the starch acetates are more thermally stable than the native starch. The starch esters showed 50% weight loss at temperatures from 328°C to 372°C, while the native starch underwent 50% weight loss at 325°C. The glass transition temperature (T_g) of the starch decreased from 273°C to 226°C. The X-ray diffraction (XRD) patterns could be classified as typical of the C-type for yam starch. X-ray diffraction also showed the loss of the ordered C-type starch crystalline structure and the degree of crystallinity of starch decreased from 36.10% to 10.96% with the increasing DS. The scanning electron microscopy (SEM) suggested that the most of the starch granules disintegrated with many visible fragments with the increasing DS.

yam, starch acetate, physicochemical characterization

1 Introduction

Chinese yam has been used as an important invigorant in traditional Chinese medicine (TCM) for many years. There are many chemical components contained in the yam such as mannan, phytic acid, amino acids, gluco-protein, cholesterol, saponins, non-starch polysaccharide, etc.^[1]. It can invigorate the spleen and stomach, promote production of the body fluids, benefit the lung and strengthen the kidney functions^[2]. There are many *Dioscorea opposita* Thunb. cultivars planted in China especially in Henan Province^[3]. In the rhizome of these *Dioscorea opposita* Thunb. cultivars, starch is the main

component making up to 20%–60% in the total biomass^[4]. However, starch is usually discarded during the extraction and isolation of the small-molecular bioactive ingredients, resulting in the waste of resources and contamination of the environment. Meanwhile, extensive research has been conducted on the structure and functional properties of the commercial starches obtained

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from crops due to their ready availability and their extensive utilization in food and non-food applications^[5]. However, the starch from medicinal plants has not been paid enough attention by starch researchers. In order to widen the application of yam and provide a new starch for the food and pharmaceutical industries, the effort should be made to study the starch in TCM.

As it is well known, native starch has the disadvantages of hydrophilicity, poor mechanical properties and dimensional stability, especially in the presence of water and in humid environments^[6]. Starch modification can overcome these shortcomings by means of altering the structure and affecting the hydrogen bonding of amylose and amylopectin in a controllable manner to enhance and extend starch application. Following crosslinking, esterification and etherification are the second most important modifications in the starch industry^[7]. The most typical starch ester is acetylated starch, which is actively marketed due to the specific properties arising from the substitution groups. The introduction of acetyl groups interrupts the ordered structure of the native starch and interferes with the re-association of amylose and amylopectin in gelatinized starch, leading to the change in morphological properties, crystalline properties, gelatinization properties involving transition temperatures and gelatinization enthalpy of the starch^[8]. Starches having a DS of 0.01–0.2 are of commercial interest because of their usage based on properties with respect to film forming, binding, adhesion, thickening, stabilizing and texturing. Highly acetylated starch with a DS of 2–3 was of research interest from 1950 through 1980 for their solubility into acetone and chloroform and for their thermo plasticity^[9]. As a biodegradable carbohydrate polymer material, acetylated starch also has many potential uses in pharmaceutical applications^[10]. Therefore, acetylated starch can have potential uses in food and pharmaceutical industries.

The purpose of this study was to understand the changes of yam starch in physicochemical properties including the morphology, crystalline structure and thermal properties after esterification. With the obtained result, it is possible to precisely evaluate the availability and provide sufficient parameters to all kinds of uses of yam starch. The physicochemical properties of yam starch were investigated by IR, SEM, XRD, DSC and TGA, respectively.

2 Materials and methods

2.1 Materials

The Chinese yam (*Dioscorea opposita* Thunb.) flour was provided by Henan Wan Xi Pharmaceutical Company. Acetic acid, acetic anhydride and sulfuric acid were purchased from Yuan Li Chemical Company. (Tianjin, China). Distilled water was used throughout this study.

2.2 Starch isolation and preparation of starch acetates

The yam flour was washed and sieved with a 150 μm mesh sifter. After depositing, the supernatant was removed by suction and the settled starch layer was resuspended in distilled water. After seven or eight cycles of depositing and resuspending repeatedly, the slurry containing starch was centrifuged at 3000 r/min for 20 min. The supernatant was discarded and the upper non-white layer was removed. Ethanol (70%) was added in order to remove the small-molecular compounds. The white layer was resuspended in distilled water and recentrifuged 3–5 times. The slurry was sieved with a 75 μm mesh sifter several times. The starch suspension obtained was dried in a convection oven at 50°C until weight constancy. The dried material was milled and sieved with a 75 μm screen to get the starch flour^[11].

The dry starch of 10 g was stirred with 10 mL of glacial acetic acid at room temperature for about 2 min. Then 30 mL of cold acetic anhydride was added over a period of five minutes. Sulfuric acid (98%) of 0.38 mL as the catalyst was diluted with 10 mL of glacial acetic acid and was added to the reaction system over a period of ten minutes. The temperature was controlled at 70°C, and then held at this temperature for 90, 120, 150 and 180 min to obtain starch acetates with DS of 0.06, 1.61, 2.28, and 2.95, respectively. When the reaction was completed, the hot starch acetate solution was added to distilled water to terminate the reaction. The ratio of reaction solution to water was maintained at about 1:10. The precipitated product was collected on a Buchner funnel, washed by distilled water to neutrality, transferred to a glass jar and dried at 50°C in a vacuum oven overnight^[12].

2.3 The determination of the degree of substitution

Determination of DS by titration involved complete basic hydrolysis of the ester linkages and titration of the

excess alkali^[13]. The starch acetate grounded sample of 0.5 g was accurately weighted and dissolved in acetone (25 mL) then an exact amount of aqueous solution of sodium hydroxide (0.5 mol/L, 25 mL) was added and the solution was stirred for 1 h at room temperature. After the indicator (phenolphthalein) was added, the excess of alkali was titrated with 0.5 mol/L hydrochloride acid. The reference sample and duplicates were treated in a similar way.

The acetyl content ($A\%$) was calculated according to the following equation:

$$A\% = [(V_0 - V_n) \times N \times 43 \times 10^{-3}] / M \times 100\%, \quad (1)$$

where V_0 in mL is the volume of 0.5 N HCl used to titrate the blank; V_n in mL is the volume of 0.5 N HCl used to titrate the samples; N is the concentration of the used HCl (mol/L); M in g is the amount of dry starch acetate sample; number 43 is the formular weight of acetyl groups. The acetyl content ($A\%$) was used to calculate the degree of substitution, DS, according to the following equation:

$$DS = 162 \times A / [4300 - (42 \times A)], \quad (2)$$

where 162 is the molecular weight of glucose units and 42 is the formular weight of acetyl group-1.

2.4 Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectra of the native starch and starch acetates were recorded with an IR spectrometer (Bruker Vector 22) using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr in a ratio of 1:30.

2.5 Scanning electron microscopy (SEM)

The morphological features of the native starch and starch acetates of different DS were observed with a scanning electron microscope (ESEM Philips XL-30). The dried samples were mounted on a metal stub and sputtered with gold to make the sample conductive, and the images were taken at an accelerating voltage of 20 kV. Micrographs were recorded at 500× magnification to ensure clear images.

2.6 X-ray diffraction studies

X-ray diffraction patterns of the native yam starch and starch acetates were analyzed using a Rigaku D/max 2500 X-ray powder diffractometer (Rigaku, Tokyo, Japan) with Nickel filtered Cu K_α radiation ($\lambda = 1.54056 \text{ \AA}$) at a voltage of 40 kV and a current of 200 mA. The scattered radiation was detected in the angular range of

$5^\circ - 60^\circ$ (2θ), with a scanning speed of 8° (2θ)/min and a step of 0.06° (2θ).

2.7 Determination of the degree of crystallinity

The degree of crystallinity of samples was quantitatively estimated by following the method of Nara and Komiya^[14]. A smooth curve, which connects the lower points in between the peaks, was computer-plotted in the diffractograms (Figure 1). The area above the smooth curve can be considered as the crystalline portion, and the lower area between the smooth curve and the linear baseline which cover the 2θ range from 4° to 30° is taken as the amorphous section. The upper diffraction peak area and the total diffraction area over the diffraction angle of $4^\circ - 30^\circ$ were integrated using the Smadchrom software (Morgan and Kennedy Research, Australia). The ratio of the upper area to the total diffraction area is used as the degree of crystallinity. The equation for calculating the degree of crystallinity is as follows:

$$X_c = A_c / (A_c + A_a),$$

where X_c refers to the degree of crystallinity; A_c refers to the crystallized area on the X-ray diffractogram; A_a is the amorphous area in the X-ray diffractogram.

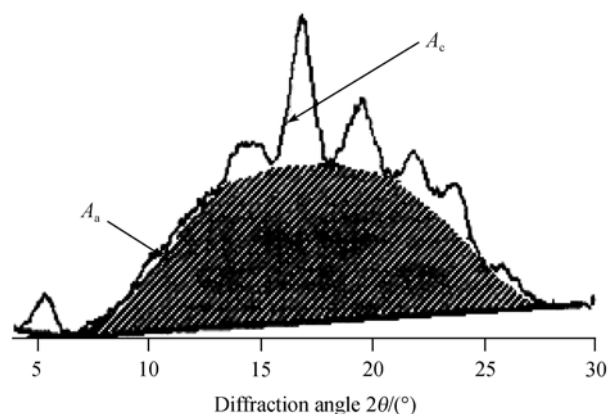


Figure 1 Calculation of degree of crystallinity of the starch.

2.8 Differential scanning calorimetry (DSC)

The thermal analyses of starch samples were carried out using a differential scanning calorimeter (DSC 204 HP, Netzsch, Germany). About 10 mg of the dried, ground sample was placed in the stainless steel DSC pans. The sample was heated from 20 to 300°C at a rate of $10^\circ\text{C}/\text{min}$ in a nitrogen atmosphere. The glass transition temperature (T_g) and melting point temperature (T_m) were recorded as the inflection point of the increment of specific heat capacity and as the peak value of the endothermal process in the DSC curves, respectively.

2.9 Thermogravimetry analysis (TGA)

The thermogravimetric measurements were performed with a TG apparatus (Shimadzu TGA-50, Kyoto, Japan). Samples of about 10 mg were heated from 20 to 500°C at a rate of 10°C/min and a flow rate of 25 mL/min in a nitrogen atmosphere.

3 Results and discussion

3.1 FT-IR

The formation of starch acetates was confirmed by the FT-IR spectra (Figure 2). Esterification resulted in the presence of a carbonyl signal in the FT-IR spectra around 1750 cm⁻¹. The intensity of the carbonyl group peak at 1750 cm⁻¹ and the intensity of the ester group peaks at 1240 cm⁻¹ and 1375 cm⁻¹ increased with the increase in DS due to the esterification. The hydrogen-bonded hydroxyl groups contribute to an extremely broad band at 3400 cm⁻¹, and the intensity of the hydroxyl groups at 3400 cm⁻¹ decreased as the increase in DS owing to the reduction in the number of hydroxyl groups.

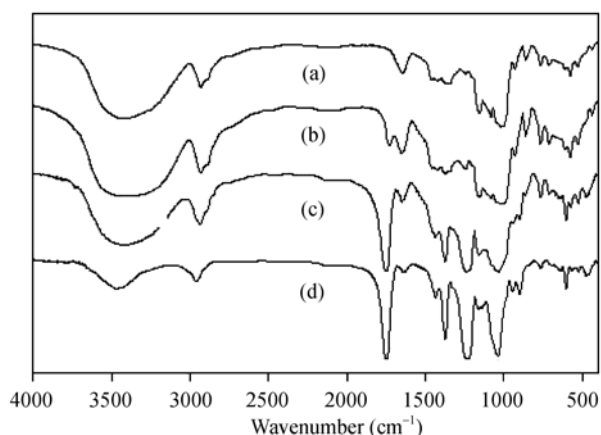


Figure 2 FT-IR spectra of native starch (a), starch acetate with a DS of 0.06 (b), starch acetate with a DS of 1.61 (c) and starch acetate with a DS of 2.95 (d).

3.2 SEM

Most of the yam starch granules were of oval to ellipsoid in shape with a few spherical ones. The starch granules lost their individuality and smoothness after esterification as a result of substitution of hydroxyl groups. As the DS of the starch acetates increased, there was an increasing destruction degree on the surface of the starch granules. As the starch acetates reached the higher degree of substitution, the starch almost fell into pieces, which reveals that the esterification not only

happened on the surface but also happened in the inner structure of the starch. Once the reaction happened, the starch became looser and more porous, which accelerated the esterification to make the starch acetate granules become small fragments (Figure 3).

3.3 XRD

The native yam starch powder had a typical C-type crystalline structure, as shown in Figure 4 with a strong diffraction peak at around 17.09° (2 θ) and a few small peaks at around 2 θ of 5.68°, 14.99°, 22.25°, 24.09° and 27.01°. Of all the diffraction peaks, the peaks at around the 2 θ of 5.68° are characteristic of the B pattern. At 2 θ of 27.01°, only one peak appeared which was indicative of the A pattern. Thus, yam starch can be classified as the C-type, which is a mixture of the A-type and B-type. In the patterns of XRD, the starch acetates showed broad peaks at 17°, 20°, 22° and 27°. The degrees of crystallinity decreased from 36.1% to 10.96% with the increase in DS. The intra and intermolecular hydrogen bonds are responsible for the highly ordered crystalline structure^[15]. On esterification, acetyl groups replace some of the hydroxyl groups on starch, reducing the formation of intermolecular hydrogen bonds and thereby resulting in the destruction of the ordered crystalline structure. As the DS of the starch acetates increased, there was an increased destruction degree or even loss of the ordered crystalline structure.

3.4 DSC

DSC was used to measure the occurrence of exothermal or endothermal changes with the increase in temperature. T_g and T_m of the native starch were changed by the esterification reaction^[16]. In Figure 5, it is shown that T_g of the native starch was 273°C and that of the starch acetate with a DS of 2.95 was 226°C, which is 47°C lower than its unmodified counterpart. T_g of starch decreased with the increasing degree of acetylation. This agrees with the results of Shogren^[17]. These changes can be explained by the fact that the intermolecular hydrogen bonds, which stiffen the macromolecular chain, decrease with the partial replacement of hydroxyl groups by acetyl groups. Moreover, the increase in the free volume within the molecules due to the introduction of bulk groups that allows more molecular mobility also contributes to the reduction in T_g of starch with acetylation^[18]. The higher the DS value of the starch acetates, the greater the free volume of the molecules.

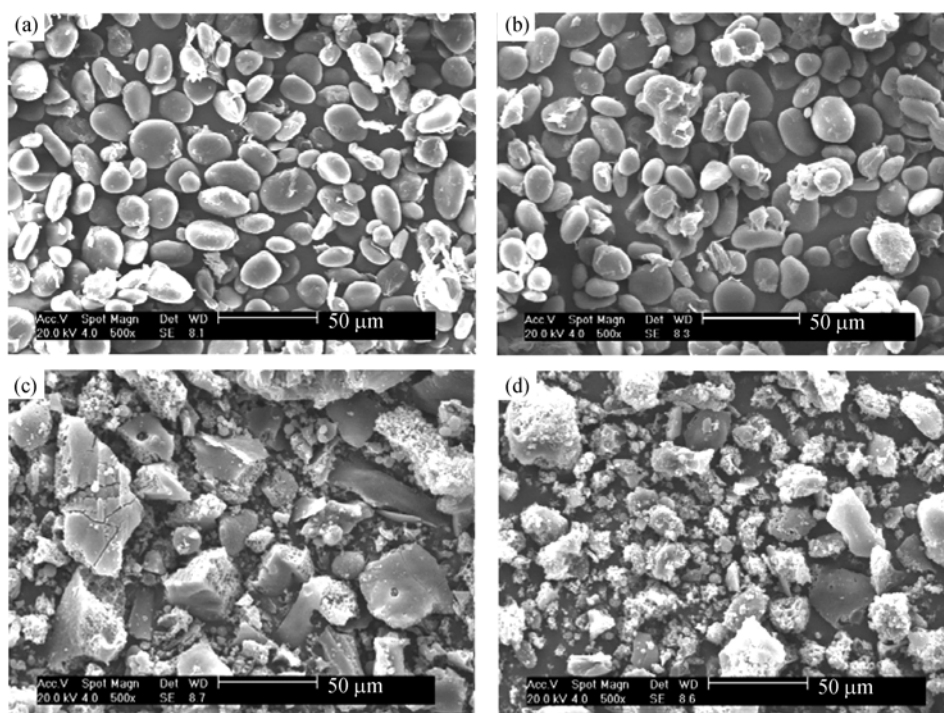


Figure 3 SEM photographs of native starch (a), starch acetate with a DS of 0.06 (b), starch acetate with a DS of 1.61 (c) and starch acetate with a DS of 2.95 (d).

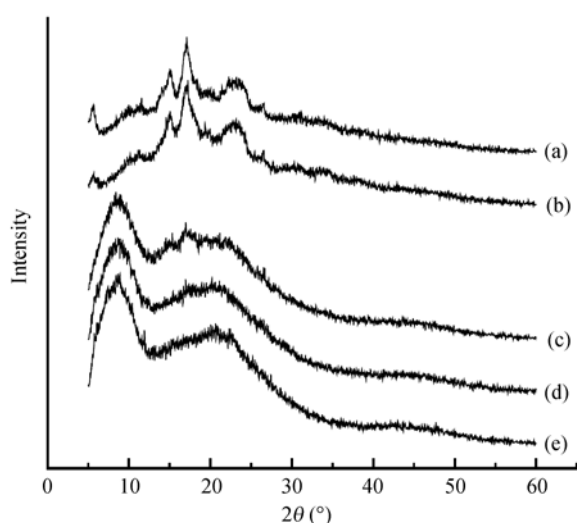


Figure 4 XRD profiles of native starch (a); starch acetate with a DS of 0.06 (b); starch acetate with a DS of 1.61 (c); starch acetate with a DS of 2.28 (d) and starch acetate with a DS of 2.95 (e).

3.5 TGA

The thermogravimetric curves were used to examine the changes in thermal stability caused by acetylation and to determine the weight loss of the material on heating. The TGA and DTG curves for the native starch and starch acetates are shown in Figures 6 and 7. The native starch showed a two-stage weight loss below 500°C, with the first minor one corresponding to the loss of wa-

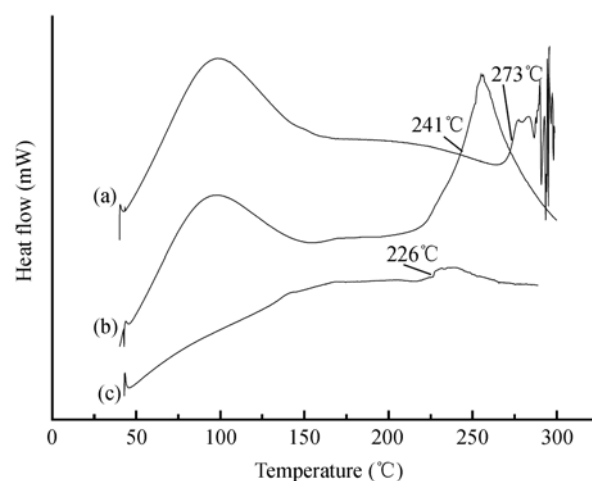


Figure 5 DSC curves of native starch (a), starch acetate with a DS of 0.06 (b) and starch acetate with a DS of 2.95 (c).

ter around 60°C–100°C and the other one corresponding to its decomposition. Water is the main product of decomposition at temperatures below 300°C. Further heating up to 500°C resulted in carbonization and ash formation^[19]. The native starch underwent 50% weight loss at 325°C while in the case of starch acetates the temperature for the same weight loss ranged from 328°C to 372°C with the increase in DS. The DTG curve for the native starch shows two peaks with the first one corresponding to the loss of water at around 60–100°C

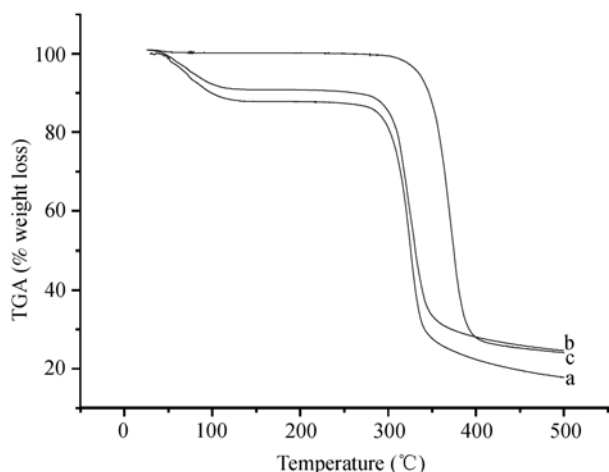


Figure 6 TGA curves of native starch (a); starch acetate with a DS of 0.06 (b) and starch acetate with a DS of 2.95 (c).

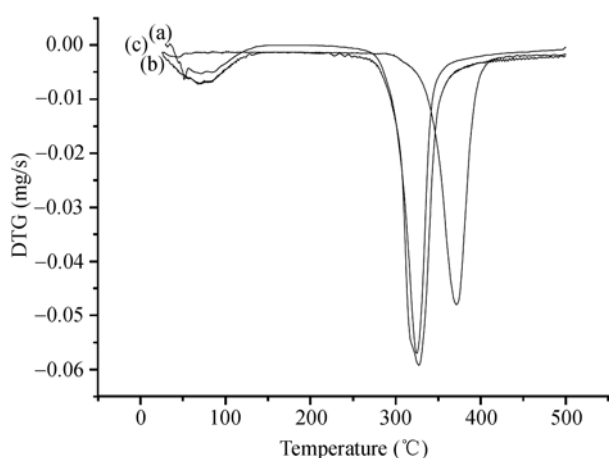


Figure 7 Derivative thermogravimetric curves of native starch (a), starch acetate with a DS of 0.06 (b) and starch acetate with a DS of 2.95 (c).

followed by a stage decomposition. There was a shifting of peak maximum towards higher temperatures with the increasing DS. The starch acetates were found to be more thermally stable than the native starch. This increase in thermal stability with the increasing DS can be

attributed to the low amount of remaining hydroxyl groups in starch molecules after acetylation. The main decomposition mechanism of starch is a result of the inter- or intra-molecular dehydration reactions of starch molecules with water as a main product of decomposition^[19], the fewer the number of hydroxyl groups remain, the better the thermal stability of the starch esters will be^[20]. Therefore, a high DS has a beneficial effect on the thermal stability of the samples.

4 Conclusions

Starch acetates with different degrees of substitution were prepared by the reaction of yam starch with acetic anhydride using sulfuric acid as the catalyst. The DS values of starch acetates could be controlled by the acetylated addition parameters such as temperature, reaction time, amount of acetic anhydride and so on. Structural modification resulted in significant changes of the physicochemical properties of the starch. The SEM images show a significant change in the shape of the starch. Most of the starch broke into pieces after esterification. The XRD patterns reveals decrease in the degrees of crystallinity and the loss of the C type starch with the increasing DS in starch acetates. The IR spectra confirm the peak intensity changes corresponding to the hydroxyl group decreases and the carbonyl groups increase with the increasing DS. Acetylation can cause a decrease in starch's glass transition temperature (T_g) but an increase in their thermal stability because the number of the hydroxyl groups in starch molecules decreases with acetylation.

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- 1 Wang S J, Gao W Y, Liu H Y. Studies on the physicochemical, morphological, thermal and crystalline properties of starches separated from different *Dioscorea opposita* cultivars. *Food Chem*, 2006, 99: 38–44
- 2 Wang S J, Gao W Y, Yu J L. The crystalline changes of starch from *Dioscorea rhizoma* by acid hydrolysis. *Chin Chem Lett*, 2006, 17: 1255–1258
- 3 Wang S J, Liu H Y, Gao W Y. Characterization of new starches separated from different Chinese yam (*Dioscorea opposita* Thunb.) cultivars. *Food Chem*, 2006, 99: 30–37
- 4 Ni S Y, Song X H. Nutritional components analysis of Chinese yam. *Jiansu Pharm Clin Res*, 2002, 10: 26–27
- 5 Kaur M, Singh N, Sandhu K S. Physicochemical, morphological, thermal and rheological properties of starches separated from kernels of some Indian mango cultivars (*Mangifera indica* L.). *Food Chem*, 2004, 85: 131–140
- 6 Xua Y X, Dzenis Y B, Hannaa M A. Water solubility, thermal characteristics and biodegradability of extruded starch acetate foams. *Ind Crop Pr*, 2005, 21: 361–368
- 7 Taggart P. Starch as an ingredient: Manufacture and applications. In A. C. Eliasson (Ed.), *Starch in food: Structure, function and applications*. Cambridge and New York: Woodhead Publishing Limited and CRC Press LLC, 2004. 363–392
- 8 Lawal O S, Adebawale K O, Ogunsanwo B M. Oxidized and acid

- thinned starch derivatives of hybrid maize: Functional characteristics, wide-angle X-ray diffractometry and thermal properties. *Intern J Bio Macromol*, 2005, 35: 71–79
- 9 Boutboul A, Giampaoli P, Feigenbaum A. Influence of the nature and treatment of starch on aroma retention. *Carbohydr Polym*, 2002, 47: 73–82
 - 10 Paronen T P, Peltonen S H, Urtti A O. Inventors. Starch acetate composition with modifiable properties, method for preparation and usage thereof. Oy Polymer Corex Kuopio Ltd, Assignee. US patent 5667803. 1997, Sep, 16
 - 11 Wang S J, Yu J L, Gao W Y. New starches from traditional Chinese medicine (TCM)-Chinese yam (*Dioscorea opposita* Thunb.) cultivars. *Carbohydr Res*, 2006, 341: 289–293
 - 12 Stefanos L, Bernice F. Synthesis of starch acetate: Statistical designed experiments to optimize the reaction conditions. *Chemometrics Intell Lab Sys*, 1997, 36: 229–243
 - 13 Rudolph S E, Glowaky R C. Preparation and properties of carboxyl-functional mixed esters of hydrolysed starch. *J Polym Sci, Polym Chem Ed*, 1978, 16: 2129–2140
 - 14 Nara S, Komiy T. Studied on the relationship between watersaturated state and crystallinity by the diffraction method for moistened potato starch. *Starch*, 1983, 35: 407–410
 - 15 Xu Y, Miladinov V, Hanna M A. Synthesis and characterization of starch acetates with high substitution. *Cereal Chem*, 2004, 81(6): 735–740
 - 16 Sagar A D, Merrill E W. Properties of fatty-acid ester of starch. *J Appl Polym Sci*, 1995, 58: 1647–1656
 - 17 Shogren R L. Preparation, thermal properties, and extrusion of high-amylose starch acetates. *Carbohydr Polym*, 1996, 29: 57–62
 - 18 Aburto J, Alric I, Thiebaud S. Synthesis, characterization, and biodegradability of fatty-acid esters of amylose and starch. *J Appl Polym Sci*, 1999, 74: 1440–1451
 - 19 Thiebaud S, Aburto J, Alric I. Properties of fatty-acid esters of starch and their blends with LDPE. *J Appl Polym Sci*, 1997, 65: 705–721
 - 20 Rudnik E, Matuschek G, Milanov N. Thermal properties of starch succinates. *Thermochimica Acta*, 2005, 427: 163–166