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Comparative studies on morphological and crystalline properties of B-type and C-type starches by acid hydrolysis

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Abstract

Comparative studies on acid hydrolysis of B-type Fritillaria starch and C-type Rhizoma Dioscorea and Radix Puerariae starches were carried out using a scanning electron microscope (SEM) and X-ray diffraction (XRD). Fritillaria, Rhizoma Dioscorea and Radix Puerariae starches were hydrolyzed with 2.2 mol/L at 35 °C for 2, 4, 8, 16 and 32 days, respectively. The SEM and XRD results revealed that B-type starch and C-type starch displayed different hydrolysis mechanisms. The acid corrosion started from the exterior surface of B-type starch granules followed by the interior core of starch granules. However, the hydrogen ion primarily attacked the interior of the C-type starch granules and then the exterior of starch granules. B-type starch granule started to crack at the hydrolysis period of 4 days while C-type starch granule was not cracked until the hydrolysis progressed up to 16 days. The crystalline type of B-type starch was not changed with increasing hydrolysis time. However, the crystalline type was gradually changed from C-type to A-type for the Rhizoma Dioscorea and Radix Puerariae starches with increase in the hydrolysis time. This result showed that the B-type polymorphs present in the C-type starch granule was preferentially hydrolyzed during the first stage of hydrolysis.

Keywords: B-type; C-type; Starch; Acid hydrolysis; Morphology; Crystallinity

1. Introduction

Starch is the major polysaccharide in plants and is in the form of granules that exist naturally within the plant cells. Starch is semicrystalline in nature with varying levels of crystallinity. Scanning electron microscope (SEM) has been used to relate granule morphology to starch genotype (Fannon, Hauber, & Bemiller, 1992). The shape of starch granules varied with the botanical source of the starch. Generally, oval, elliptic, spherical and irregular shapes predominate among all the granule shapes. X-ray powder diffraction diffactometry has been used to reveal the presence and characteristics of crystalline structure of the starch granules (Hoover, 2001). Starch can be classified to A, B, and C forms. In the native granular forms, the A-type starch was associated mainly with cereal starches, such as maize starch and wheat starch. The X-ray patterns of these kinds of starch gave the stronger diffraction peaks at around 15°, 17°, 18° and 23°. The B-type starch was usually obtained from tuber starches, such as potato starch and canna starch. The strongest diffraction peak of the X-ray diffraction pattern appeared at 17° 2θ. There were also a few small peaks at 2θ values of 20°, 22° and 24°. The C-type starch was a mixture of both A and B types, such as smooth-seeded pea starch and various bean starch (Cheetham & Tao, 1998; Elsenhaber & Schulz, 1992; Zobel, 1988a).

Acid modification is widely used in the starch industry to produce thin boiling starches for use in food, paper, textile and other industries (Rohwer & Klem, 1984). The typical procedure to manufacture acid-thinned starch

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involves treating a concentrated starch slurry at a temperatures lower than the gelatinization temperature of the starch with mineral acid for a period of time. Acid modification could change the morphological properties, crystalline properties, gelatinization properties involving transition temperatures and gelatinization enthalpy, viscoelastic properties of starch. So, acid modification of starch could be very helpful to understand the inner structure of starch granules (Kang, Kim, Lee, & Kim, 1997; Kim & Ahn, 1996; Lawal, Adebowale, Ogunsanwo, Barba, & Ilo, 2005; Olayide, 2004; Shi & Seib, 1992; Virtanen, Autio, Suortti, & Poutanen, 1993).

Many researchers have reported structures and physical properties of A-type and B-type starch as affected by acid hydrolysis. The SEM results showed that the acid firstly attacked the surface of the granules and then the interior (Cowie & Greenwood, 1957; Wang, Truong, & Wang, 2003; Komiya & Nara, 1986; Komiya, Yamada, & Nara, 1987; Wang & Wang, 2001). Acid hydrolysis could not change the crystalline type of A-type and B-type starch. However, there was little information on acid hydrolysis of the C-type starch (Wang, Gao, Yu, & Xiao, 2006; Wang, Yu, Yu, Chen, & Pang, in press). Starches separated from different Fritillaria, cultivars of Chinese Yam (*Dioscorea opposita* Thunb.) and Radix Puerariae have been investigated for physico-chemical (amylose content, swelling power, solubility, light transmittance, water binding capacity and turbidity), morphological (including shape and size), thermal, and crystalline properties in our previous study. These starches from different cultivars showed different properties (Wang et al., 2005; Wang et al., 2006; Wang et al., 2006; Wang, Gao et al., 2006; Wang, Liu et al., 2006; Wang et al., 2006). In this study, the morphological and crystalline changes of B-type Fritillaria starch and C-type Rhizoma Dioscorea and Radix Puerariae starches during acid hydrolysis were compared by SEM and XRD.

2. Materials and methods

2.1. Materials

Fritillaria thunbergii starch was isolated in our laboratory (Wang et al., 2005). *F. thunbergii* was washed and comminuted to a powder using a plant micro-muller (FZ102, Taisite Instrument Ltd., Tianjin, China) which were sieved using 160 mesh sifter and subsequently kept in a desiccator. The dried powders were extracted with 95% ethanol by cold immersion method for 24 h. The supernatant was removed and the sediment was re-suspended in 95% ethanol for 24 h again. The resulting suspension was filtrated with a G4 type anti-acid filter. The residue was washed with 95% ethanol several times and then suspended in distilled water a number of times until the supernatant became transparent. The starch was then collected and dried at room temperature.

D. opposita Thunb. cv. Baiyu starch was obtained from dried Rhizoma of *D. opposita* Thunb. cv. Baiyu in our laboratory (Wang et al., 2006). The dried *D. opposita* Thunb. was washed, cut into small pieces and ground with a plant micro-muller and then sieved using a 100 mesh sifter. After sieving, the *D. opposita* Thunb. powders were immediately steeped in water containing 0.1% $HgCl_2$ to prevent the microbial growth. After depositing, the supernatant was removed by suction and the settled starch layer was resuspended in distilled water. After precipitating and resuspending seven or eight times, the slurry containing starch was centrifuged in wide-mouthed cups at 3000 r/min (rotator size, 6 cm) for 10 min. The supernatant and upper non-white layer such as cellulose were removed. The white layer (starch layer) was resuspended in distilled water and recentrifuged for 3–5 times. The starch was then collected and dried at room temperature automatically.

The isolation method of Radix Puerariae starch (RPS) was the same as that of Rhizoma Dioscorea starch.

2.2. Preparation of acid-thinned starch

The native starch (2 g, on a dry weight basis) was hydrolyzed by suspending in 80 ml of 2.2 mol/L HCl solution at 35 °C for 2, 4, 8, 16 and 32 days without stirring. After hydrolysis, the suspension was filtrated by G4 type anti-acid filler under low pressure. The filter cake was washed several times with distilled water until the pH value of the filtrate was 7. The resulting filter cake was washed 2–4 times with acetone again. The resulting acid-thinned starch was dried at the room temperature overnight (air stream) and utilized throughout the whole experiment.

2.3. Morphological properties

Scanning electron micrographs were obtained with an environmental scanning electron microscope (ESEM, Philips XL-30, Holland). Acid-thinned starch samples were suspended in acetone to obtain a 1% suspension. One drop of the starch-acetone suspension was applied on an aluminum stub using double-sided adhesive tape and the starch was coated with gold powder to avoid charging under the electron beam after the acetone volatilized. An accelerating potential of 30 kV was used during micrography.

2.4. X-ray diffractometry

X-ray powder diffraction measurements were done using PANalytical X'Pert Pro diffractometer (PANalytical X'Pert PRO-MRD, Holland). The detailed operating conditions are referred to the method of Wang, Gao, Yu et al. (2006).

2.5. Determination of the degree of crystallinity

The degree of crystallinity of samples was quantitatively estimated following the method of Nara and Komiy (1983).

3. Results and discussion

3.1. Scanning electron microscopy (SEM)

The action pattern of various acids on B-type granular starches has been investigated earlier (Liu, 2003; Sun, Yu, & Liu, 2004a; Sun, Yu, & Liu, 2004b). The previous study showed that the acid preferentially attacked the exterior of starch granules and then the interior. SEM photographs of unmodified starch and acid-thinned B-type Fritillaria starches were displayed in Fig. 1. The size of native Fritillaria starch was variable and ranged from 5 to 30 μm. The granule surface appeared smooth, round or elliptic-shaped. From the SEM photographs, it was evident that hydrolysis does not occur uniformly. Some areas are much more susceptible to attack than others. The acid acts by first attacking the surface and forming the cracks on the surface at the first hydrolysis step. Acids cause surface alternations and degrade the external part of the granule by exo-corrosion. When endo-corrosion occurs, the internal part of the granule is corroded through small cracks by which acids could penetrate the granule. In a given starch sample, small granules are more rapidly hydrolyzed than bigger ones, because of a larger available surface area. It appears that the starch granules which are readily, or rapidly, hydrolyzed with acid are those whose surfaces are readily attacked with the formation of canals. With the increase in hydrolysis time, the starch granules start to fall to pieces due to the severe corrosion. After 32 days of hydrolysis, no intact starch granules could be observed under the SEM.

The SEM photographs of C-type Rhizoma Dioscorea and Radix Puerariae starches before and after hydrolysis

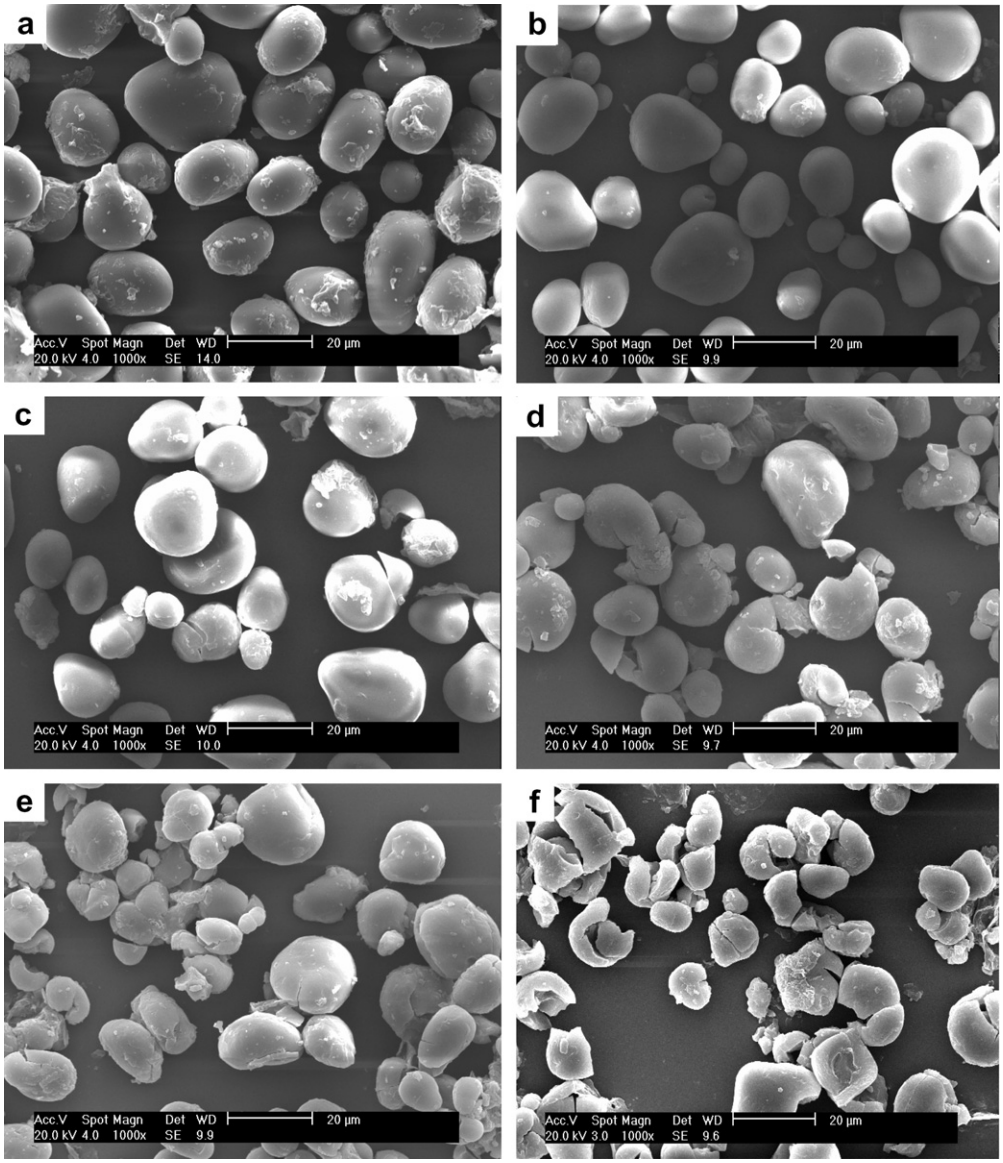

Fig. 1. SEM photographs of unmodified and modified starches: (a) unmodified starch; (b) 2 days; (c) 4 days; (d) 8 days; (e) 16 days; (f) 32 days.

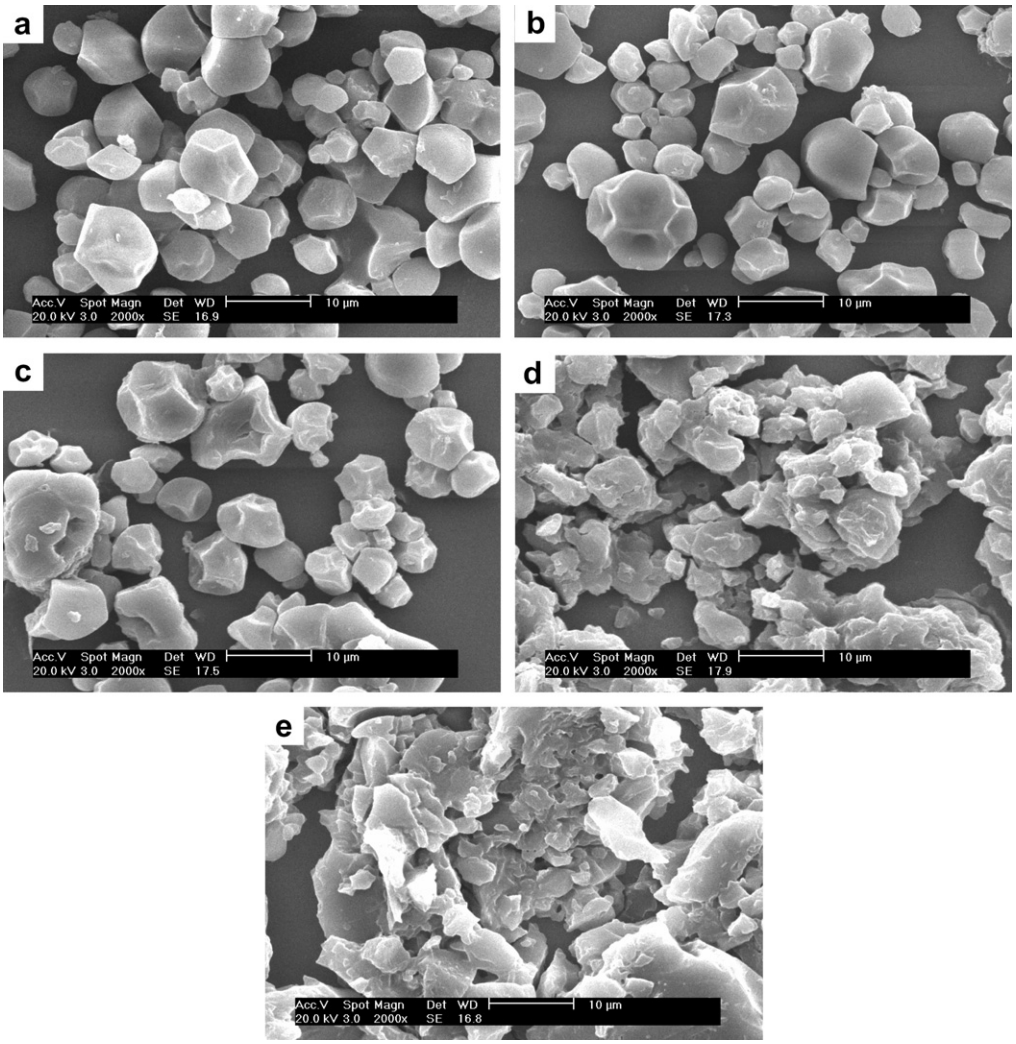

Fig. 2. SEM photographs of unmodified and modified starches: (a) unmodified starch; (b) 2 days; (c) 4 days; (d) 8 days; (e) 16 days.

are shown in Figs. 2 and 3. Native Radix Puerariae starch showed the presence of starch granules from small to large and polygonal to irregular with diameters ranges between 1–5 and 5–20 μm, respectively, for small and large granules. Native Rhizoma Dioscorea starch granules appeared oval, elliptic or spherical with sizes ranging from 5–50 μm reported in our previous study. When the two C-type starch granules were subjected to 2 days of acid hydrolysis, no obvious changes occurred from the SEM photographs. However, some concaves appeared on the surface of the acid-thinned starch granules (directed by arrows in Fig. 3b_2 and Fig. 3c_2). With the increasing in hydrolysis time (4 days), the concaves became more obvious. When the starch granules were subjected to 8 days of acid hydrolysis, the Radix Puerariae starch granules fell to small pieces which were connected each other. However, the Rhizoma Dioscorea starch granules still remained intact and smooth without any corrosions or fissures. The above results showed that the core part of the starch granule was more easily attacked by the hydrogen ion. Generally, the amorphous regions of starch granules are preferentially hydrolyzed in the process of acid hydrolysis. So, SEM results indicated that the amorphous regions were mainly located interior of starch granules. After 16 days of hydrolysis, The Radix Puerariae starch granules had been corroded completely to form some lumps under SEM observation. As for the Rhizoma Dioscorea starch, the starch granules started to fracture and some cracks came to appear on their surfaces due to heavy hydrolysis. The heaviest corrosion of Rhizoma Dioscorea starch granules occurred for 32 days of hydrolysis. Whether the granule size or shape all showed significant variations viewed under SEM. Any intact starch granules could not be found and all the fragments were conglutinated together due to the heavy acid erosion. The above results demonstrated that the amorphous regions mainly located at the centre of C-type starch granules, while the crystalline areas mainly existed exterior of the starch granules. This result was in agreement with those of Bogracheva, Morris, Ring, and Hedley (1998).

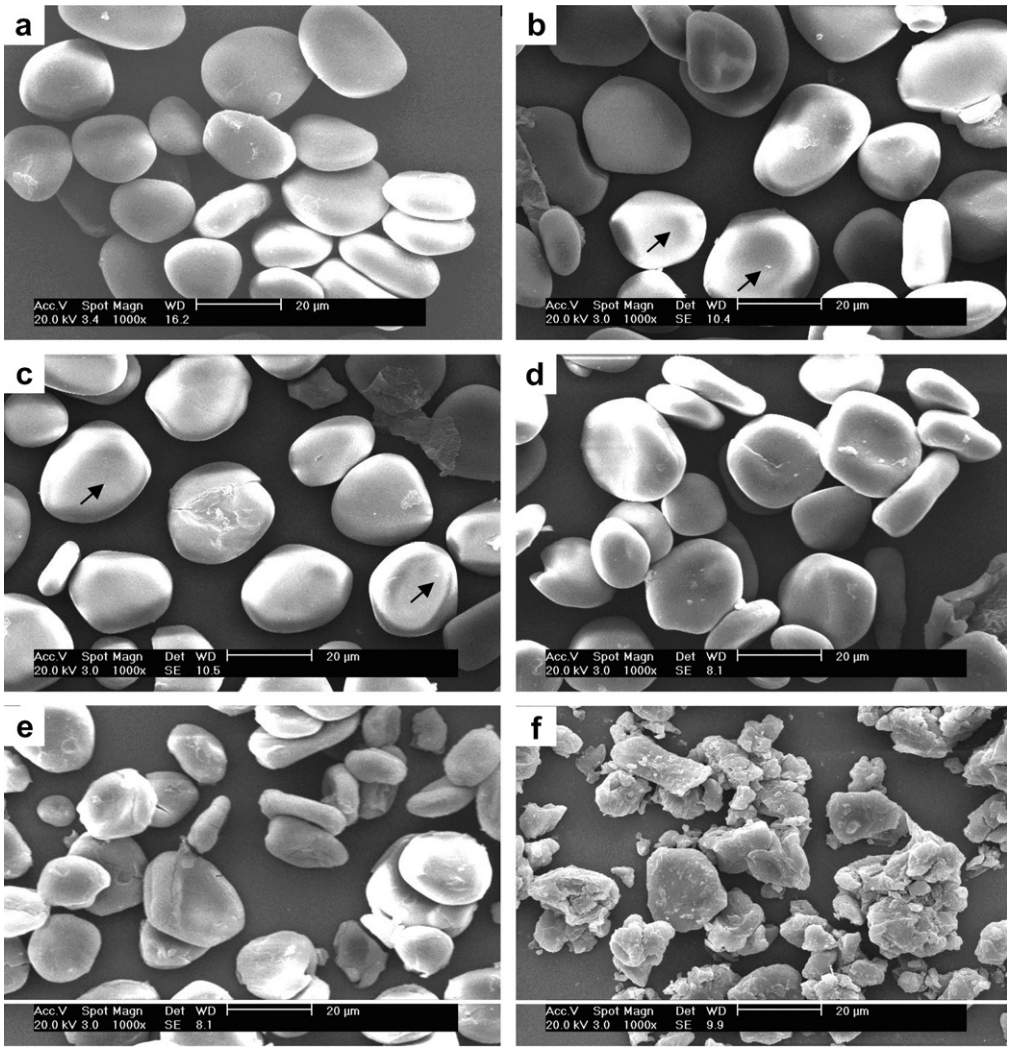

Fig. 3. SEM photographs of unmodified and modified *D. BY* starches: (a_1, a_2) unmodified starch; (b_1, b_2) 2 days; (c_1, c_2) 4 days; (d_1, d_2) 8 days; (e_1, e_2) 16 days; (f_1, f_2) 32 days.

3.2. X-ray diffraction

The X-ray diffraction patterns of B-type Fritillaria starch and C-type starch before and after hydrolysis are shown in Figs. 4a–4c.

As reported in our early study, Fritillaria starch showed the typical B-type X-ray diffraction pattern. Acid hydrolysis did not change the crystalline type of B-type starch. Whether it was subjected to hydrolysis for 2 days or 32 days, the crystalline type of acid-thinned starch still remained typical B-type diffraction pattern. Native Rhizoma Dioscorea and Radix Puerariae starches display characteristic of typical C-type diffraction pattern. The peak at around 2θ value of 6.5° were characteristic of B-type pattern, while at 27.4° 2θ were indicative of the A-type pattern. With increasing acid hydrolysis time, the crystalline type of these two C-type starches changed from C-type to A-type gradually. As for Radix Puerariae starch, the crystalline type changed from typical C-type pattern to typical A-type pattern.after 2 days of acid hydrolysis. The peak at around 2θ value of 6.5° disappeared. However, the Rhizoma Dioscorea starch changed from typical C-type pattern to typical A-type pattern after 16 days of acid hydrolysis. Except the peak at around 2θ value of 6.5° disappearing, the peak at 20.0° 2θ was split into two small peaks at 20.0° 2θ and 21.0° 2θ which also indicated that the B-type polymorph has been hydrolyzed heavily. That is to say, the A-type polymorphs became the major component in the acid-thinned starch. This result was much different from that of other acid-thinned starches which exhibited the same crystalline type comparison with the unmodified starch (Atichokudomchai & Varavinit, 2003; Lawal et al., 2005; Olayide, 2004; Wang et al., 2003).

The X-ray diffraction results revealed that the B-type polymorphs in the C-type starch was firstly degraded during the acid hydrolysis. Generally, amorphous areas were firstly hydrolyzed followed by the crystalline

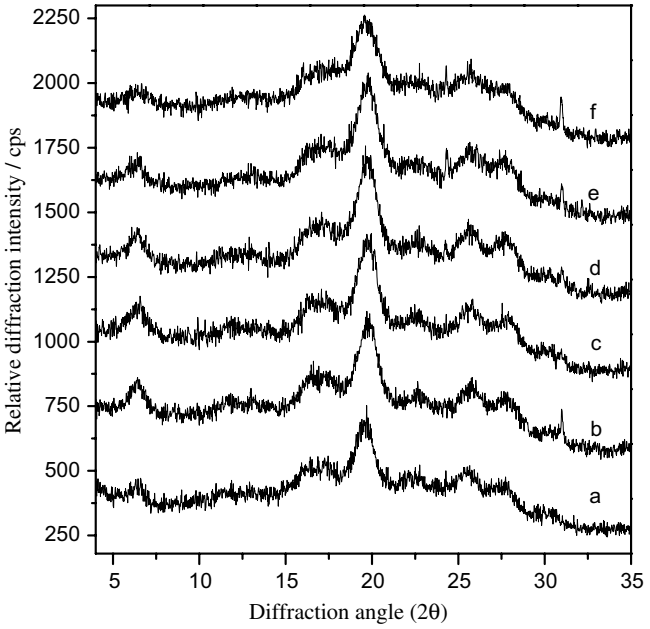

Fig. 4a. X-ray diffraction spectra of unmodified and acid-thinned *D. BY* starches: (a) 0 day; (b) 2 days; (c) 4 days; (d) 8 days; (e) 16 days; (f) 32 days.

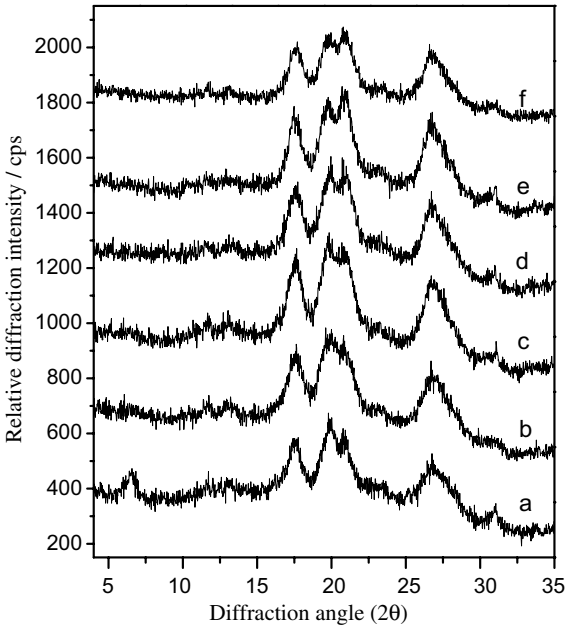

Fig. 4b. X-ray diffraction spectra of unmodified and acid-thinned RPS starches: (a) 0 day; (b) 2 days; (c) 4 days, (d) 8 days; (e) 16 days; (f) 32 days.

regions. That is to say, B-polymorphs contained in C-type starch mainly constituted the amorphous regions while the crystalline areas were primarily resulted from the A-polymorphs. From SEM and X-ray diffraction results demonstrated that the B-polymorphs existed in the centre of C-type starch granules which were surrounded by the A-polymorphs.

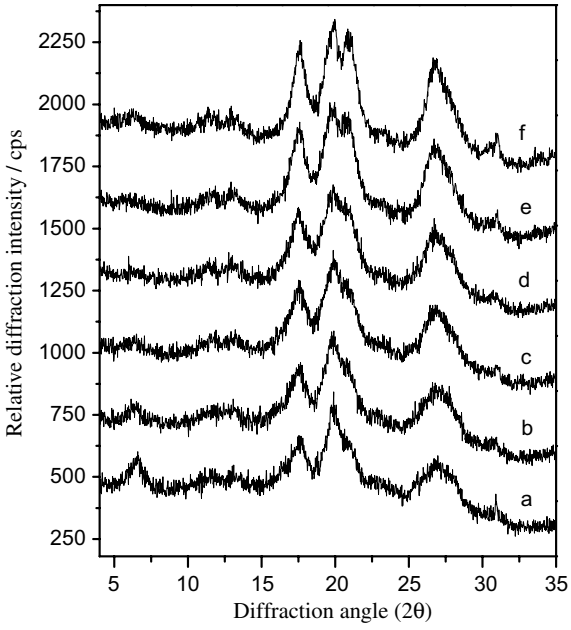

Fig. 4c. X-ray diffraction spectra of unmodified and acid-thinned *D. BY* starches: (a) 0 day; (b) 2 days; (c) 4 days; (d) 8 days; (e) 16 days; (f) 32 days.

4. Conclusions

The changes in granular structure and crystalline properties of B-type Fritillaria starch and C-type Rhizoma Dioscorea and Radix Puerariae starches were compared by acid hydrolysis. The acid preferentially attacked the surface of the B-type Fritillaria starch and then the interior of granule. However, acid first attacked the interior of the C-type Rhizoma Dioscorea and Radix Puerariae starches, resulting in the formation of concaves on the surface of the granules. After 8 and 16 days of hydrolysis for Radix Puerariae and Rhizoma Dioscorea starches, the starch granules started to crack when the interior amorphous regions was hydrolyzed completely. The SEM results of C-type starch subjected to hydrolysis showed that the amorphous regions were mainly located in the interior of granules while the exterior were mainly constituted with crystalline areas. Acid hydrolysis did not change the crystalline type of B-type Fritillaria starch. However, the crystalline type transformed from C-type to A-type with the acid hydrolyzing for C-type Rhizoma Dioscorea and Radix Puerariae starches. This revealed that the B-type polymorphs constituted the C-type starch were preferentially attacked by the acid followed by the A-type polymorph. According to SEM deduction, the B-type polymorph existed in the centre of C-type polymorph which was surrounded by the A-type polymorph.

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