Carbohydrate Polymers 74 (2008) 731–739

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

The semi-crystalline growth rings of C-type pea starch granule revealed by SEM and HR-TEM during acid hydrolysis

Shujun Wang [a,*], Jinglin Yu [b], Jiugao Yu [c]

[a] School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, China
[b] College of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China
[c] College of Science, Department of Chemistry, Tianjin University, Tianjin 300072, China

ARTICLE INFO

Article history:
Received 7 October 2007
Received in revised form 18 January 2008
Accepted 4 March 2008
Available online 18 March 2008

Keywords:
Pea starch
C-type starch
Semi-crystalline
Growth rings
Crystalline lamella

ABSTRACT

Semi-crystalline growth rings and the crystalline lamella of C-type pea starch were studied by Scanning Electron Microscope (SEM) and Field Emission Gun Transmission Electron Microscope (FEG-TEM) during acid hydrolysis. The alternating growth rings (semi-crystalline and amorphous growth rings) are clearly observed under SEM. The thickness of semi-crystalline growth rings is variable and approximately 200 nm apart. SEM results also reveals that the amorphous areas mainly locate core part of C-type starch granules, while the crystalline areas mainly exist in the peripheral region of starch granules. The crystalline and the amorphous areas are hydrolyzed concomitantly, although slowly. The clear lattice fringes with different orientation corresponding to the different thickness of crystalline lamella were observable from high-resolution-TEM (HR-TEM) images. The crystalline lamella size can be confirmed to be about 20 nm with the length of 20 nm and the thickness of 20 nm. The clear lattice fringes should correspond to the crystalline phases in the semi-crystalline growth rings while the blurry regions correspond to the amorphous growth rings among the semi-crystalline growth rings.

1. Introduction

Starch, the major energy storage in higher plants, occurs in the form of semi-crystalline birefringent granules mostly composed of two components, namely linear amylose and branched amylopectin, both polymers consisting of α-linked D-glucosyl units (Buléon, Colomna, Planchot, & Ball, 1998; Jane, Kasemsuwan, Leas, Zobel, & Robyt, 1994; Oostergetel & van Bruggen, 1989). The semi-crystallinity of starch has been established for many decades, on the basis of their X-ray diffraction patterns. These patterns indicate that there are two main allomorphs of starch: A-starch occurring essentially in cereals and B-starch in most tubers. C-starch, a third allomorph, which is believed to be the sum of A + B, is also sometimes observed in some legumes and Rhizomas (Cairns, Bogracheva, Ring, Hedley, & Morris, 1997; Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001; Wang, Gao, et al., 2006; Wang, Liu, et al., 2006; Wang, Yu, Gao, Liu, & Xiao, 2006). In terms of structural work, a number of reports have dealt with the elucidation of the molecular and crystal structure of A- and B-starches (Oostergetel & van Bruggen, 1989; Sarko & Wu, 1978; Vermeylen, Goderis, Reynaers, & Delcour, 2004). In these two allomorphs, it is believed that crystallites consists of parallel, left-handed double helices formed from the short chains of the amylopectin molecules. In A- and B-starches, these crystallites are thought to have the same double helical conformation but different packing arrangement and different intra-crystalline water content. The case of C-starch is more complex as its X-ray diffraction diagram can be resolved as a combination of A- and B-crystalline constituents, the amount of each of these depending on the sample origin.

Starch is considered to be structured on five different length scales: the whole granule architecture (2–100 μm), growth rings (120–500 nm), blocklets (20–500 nm), amorphous and crystalline lamellas (9 nm), amylopectin and amylose chains (0.1–1.0 nm) (Vandeputte & Delcour, 2004). French (1984) suggested that the semi-crystalline growth rings with 16 repeating clusters have a thickness between 120 and 400 nm. Cameron and Donald (1992) suggested that the relatively wide radial growth rings in the starch granules consist of semi-crystalline shells with about 140 nm of thickness which was partitioned by the amorphous growth rings at least as thick as the semi-crystalline one. Based on the AFM measurements, Dang and Copeland (2003) suggested the growth rings in rice are approximately 400 nm apart. One semi-crystalline growth ring (120–400 nm thick) consists of a stack, i.e. about 16 repeats, of alternating amorphous (2–5 nm thick) and crystalline (5–6 nm thick) lamellae (Cameron & Donald, 1992; French, 1984).

The exact location of the A- and B-components in C-starch has been debated for a long time. Quite recently, in a study on smooth pea starch – the best known C-starch specimen – it was shown that

* Corresponding author.
E-mail address: shujunwy@tju.edu.cn (S. Wang).

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.03.001

these granules dispersed into dilute aqueous KCl underwent a stepwise gelatinization when the temperature of the gelatinizing medium was increased (Bogracheva, Morris, Ring, & Hedley, 1998). At around 65 °C only the inner part of the granule became swollen whereas the outer part remained intact until around 75 °C where this part started also to gelatinize. By comparing this behavior with that of standard A- and B-starches, the authors of this study concluded that the centre of the granules consisted mainly of the B-allomorph whereas the A-phase was located toward the outside of the granules. Structural changes of C-type Rhizoma *Dioscorea* starch during acid hydrolysis also reveals that the B-type polymorph exist in the core of C-type starch granule which is surrounded by the A-type polymorph. Amorphous regions are essentially composed of B-type polymorph while A-type polymorph chiefly constitutes the crystalline regions (Wang et al., 2007a; Wang, Yu, Yu, Chen, & Pang, 2007b). But so far, there are few reports on the semi-crystalline growth rings of C-type pea starch (Ridout, Parker, Hedley, Bogracheva, & Morris, 2004a; Ridout, Parker, Hedley, Bogracheva, & Morris, 2004b). The present work was undertaken to see the growth rings and crystalline lamella of C-type smooth starch during acid hydrolysis with the use of SEM and TEM.

2. Experimental section

2.1. Acid hydrolysis

A sample of smooth pea starch was kindly supplied from Nutri-Pea Limited, ACCU-GEL (Canada). The acid-modified starch was prepared according to the methods in our previous reports (Wang et al., 2007a, 2007b). A quantity of 25 g (dry basis) of native pea starch was hydrolyzed by suspending in 500 ml of 2.2 mol/L HCl solution at 35 °C for 2, 4, 8, 12, 16, 20 and 25 days with stirring for 2 times a day. After hydrolysis, the suspension was filtered with a G4-type acid funnel by vacuum filtration. The insoluble residue was washed several times with distilled water to neutrality. The resulting filter cake was again washed 2–4 times with acetone for dehydration. The resulting acid-thinned starch was dried at the room temperature overnight (air stream).

The hydrolysis yield (wt %) was calculated as the following equation:

$$\text{Hydrolysis yield (wt \%)} = W_a/W \times 100\%,$$

W_a: weight of starch (dry basis) after acid hydrolysis.
W: weight of starch (dry basis) before acid hydrolysis.

2.2. SEM observations

The granular structure changes were analyzed by using an environmental Scanning Electron Microscope (ESEM, Philips XL-3, Holland) at 20 kV. Acid-thinned starch samples were suspended in acetone to obtain a 1% suspension. One drop of the starch–acetone suspension was deposited on a slide and dried automatically. The samples were coated with gold powder to avoid charging under the electron beam.

2.3. FEG-TEM observations

Specimen was suspended in anhydrous ethanol for 2–3 min sonification to obtain a dilute and homogeneous suspension. A drop of dilute suspension was deposited onto a glow discharged carbon-coated microscopy grid and allowed to dry. Once positioned in to a Gatan 626 specimen holder, they were quench-frozen in liquid nitrogen, transferred into the microscope, and observed at low temperature (−180 °C). All observations were performed with a TECNAI Field Emission Gun Transmission Electron Microscope (FEG-TEM, Philips Tecnai G^2 F20, Holland) operated at 200 kV for conventional imaging and high-resolution imaging. All recordings were achieved with an image intensified CCD camera with video frequency readout, online diditalization, and 16 bit frame accumulation. The CCD camera was positioned 50 mm from the sample.

2.4. X-ray diffraction analysis

The X-ray diffraction pattern was recorded with a cobalt anode X-ray tube (Co–K_α radiation, $\lambda = 0.178901$ nm) using a Panalytical X'Pert Pro powder X-ray diffractometer (PANalytical, Holland). The starch powders were packed tightly in sample holders. Each sample was exposed to the X-ray beam at 45 kV and 30 mA. The scanning region of the diffraction angle (2θ) was from 4° to 35° at 0.0170° step size with a scan step time of 30.8451 s. All the specimens were stored in a desiccator where a saturated solution of NaCl maintained a constant humidity atmosphere (relative humidity RH = 75%) atmosphere for one week before measurements. The degree of crystallinity of samples was quantitatively estimated following the method of Nara and Komiy (1983).

3. Results and discussion

3.1. Recovery yield

Fig. 1 shows the hydrolysis yield curve of pea starch. Yield of acid-modified starches is decreasing with the increase in acid hydrolysis time. The hydrolysis process of 25 days duration could be divided into three steps, the first one being from 0 to 8 days, the second from 8 to 16 days and the last one from 16 to 25 days. The first two stages have higher hydrolysis rate which is mainly attributed to the hydrolysis of amorphous material. The third phase with slow hydrolysis rate is ascribed to the hydrolysis of crystalline region. Acid attacks amorphous areas more rapidly than the crystalline ones, less accessible to H_3O^+ ions (Jayakody & Hoover, 2002; Jenkins & Donald, 1997). Potato starch also shows three-stage profile in which the first two stages have the higher hydrolysis rate due to degradation of amorphous regions. The third-stage with slow hydrolysis rate is ascribed to the crystalline phase

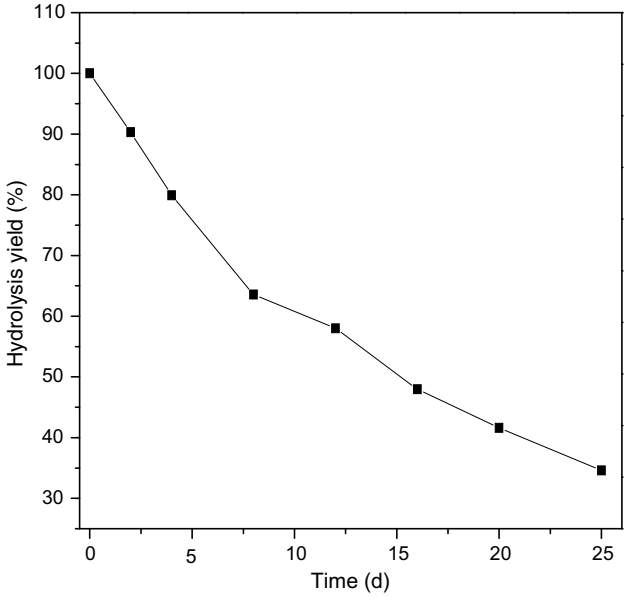

Fig. 1. Yield of starch after acid hydrolysis.

(Wang & Wang, 2001). However, corn or waxy maize starch give the two-stage profile of the hydrolysis (Putaux, Molina-Boisseau, Momaur, & Dufresne, 2003). A relatively fast hydrolysis (corresponding mainly to the degradation of amorphous region) is followed by a slow rate, corresponding mainly to the degradation of crystalline regions.

3.2. SEM

SEM photographs of native starch and acid-modified starches are displayed in Fig. 2. The granule size of native pea starch is variable and ranges from 3 to 40 μm. Two distinct populations in the size distribution of pea starch granules (large granules with diam-

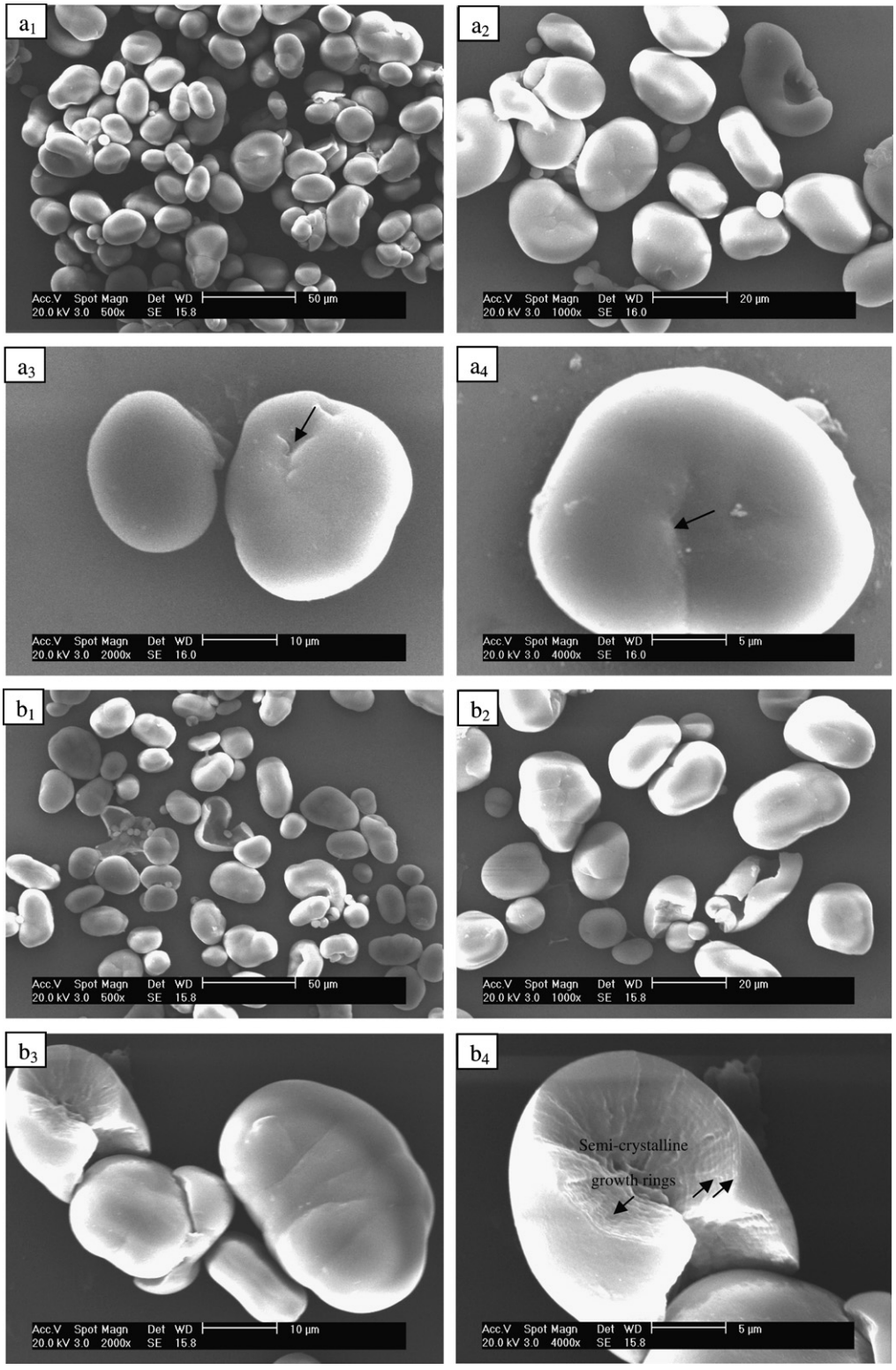

Fig. 2. SEM photographs of native and modified starches. (a) Native starch; (b) 2 days; (c) 4 days; (d) 8 days; (e) 12 days; (f) 16 days; (g) 20 days; (h) 25 days.

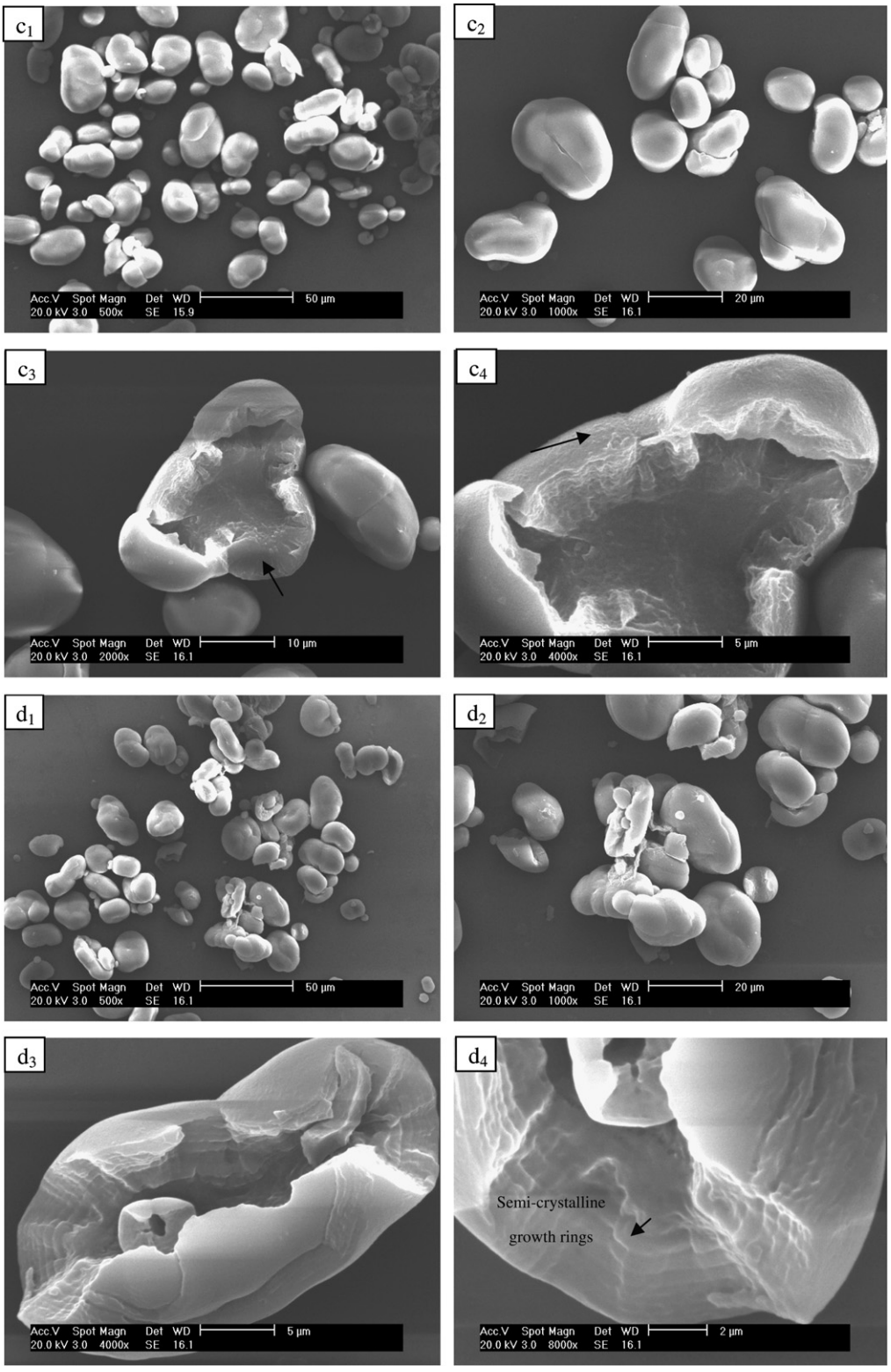

Fig. 2 (*continued*)

eters ranging from 15 to 40 μm and small granules with diameters ranging from 3 to 8 μm) could be found. Most of the granules are oval, although spherical, round, elliptical and irregularly shaped granules are also found in Fig. 2a_1. The surfaces of most of starch granules are not smooth enough with the evidence of some wrinkles (arrows in Fig. 2a_3 and 2a_4). After 2 days of hydrolysis, the surface of most of the starch granules is still smooth but some of starch granules are cracked and imperfect (Fig. 2b_1, b_2, b_3 and b_4). The alternating growth rings (protrusions indicate semi-crystalline growth rings among which amorphous regions are arrayed regularly) are vaguely seen at 4000× magnification in Fig. 2b_4. The thickness of semi-crystalline growth rings is variable and approximately 200 nm apart. Acid corrosion from exterior to interior results in the fracture of starch granules which reveals that starch

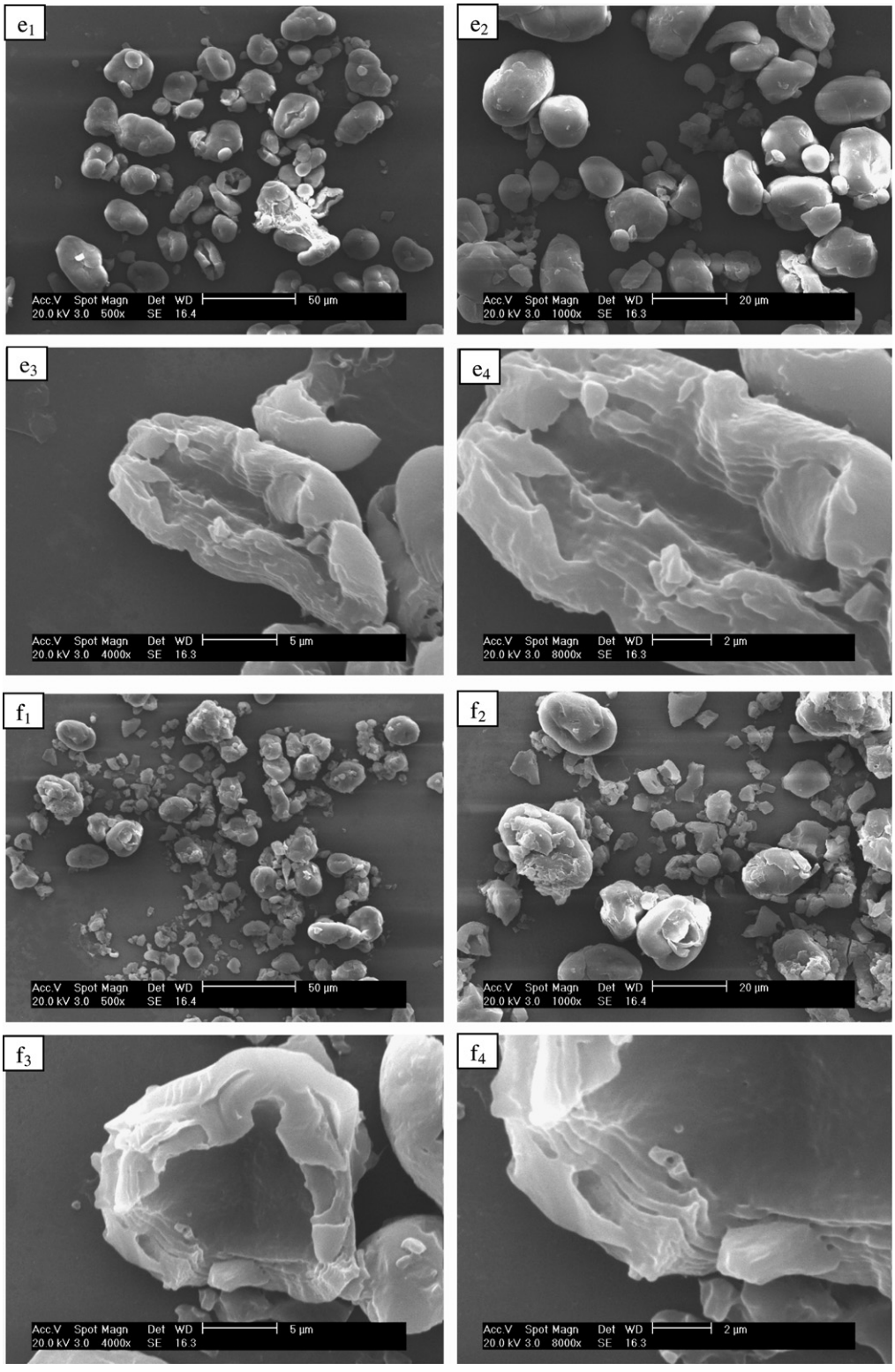

Fig. 2 (*continued*)

granules are substantial. A slightly roughened surface due to exocorrosion is observed for the starches granules hydrolyzed for 4 days (Fig. 2c_1 and c_2). The semi-crystalline growth rings are still visible from SEMs (Fig. 2c_3 and c_4). In addition, the interior of starch granules is also corroded and some empty. At 8 days of hydrolysis, severe exocorrosion has taken place all over the granule surface (Fig. 2d_3). The outer layer (or upper layer) of starch granules is partly eroded away from the surface. The semi-crystalline growth rings are concomitantly hydrolyzed, although slowly. This proves that the degradation of crystalline regions is simultaneous with that of the amorphous areas. The disruption of the semi-crystalline growth rings starts as soon as the acid can diffuse in the inside of starch granules. After prelonged hydrolysis for 12 days, the outer layer of some of starch granules has been eroded entirely.

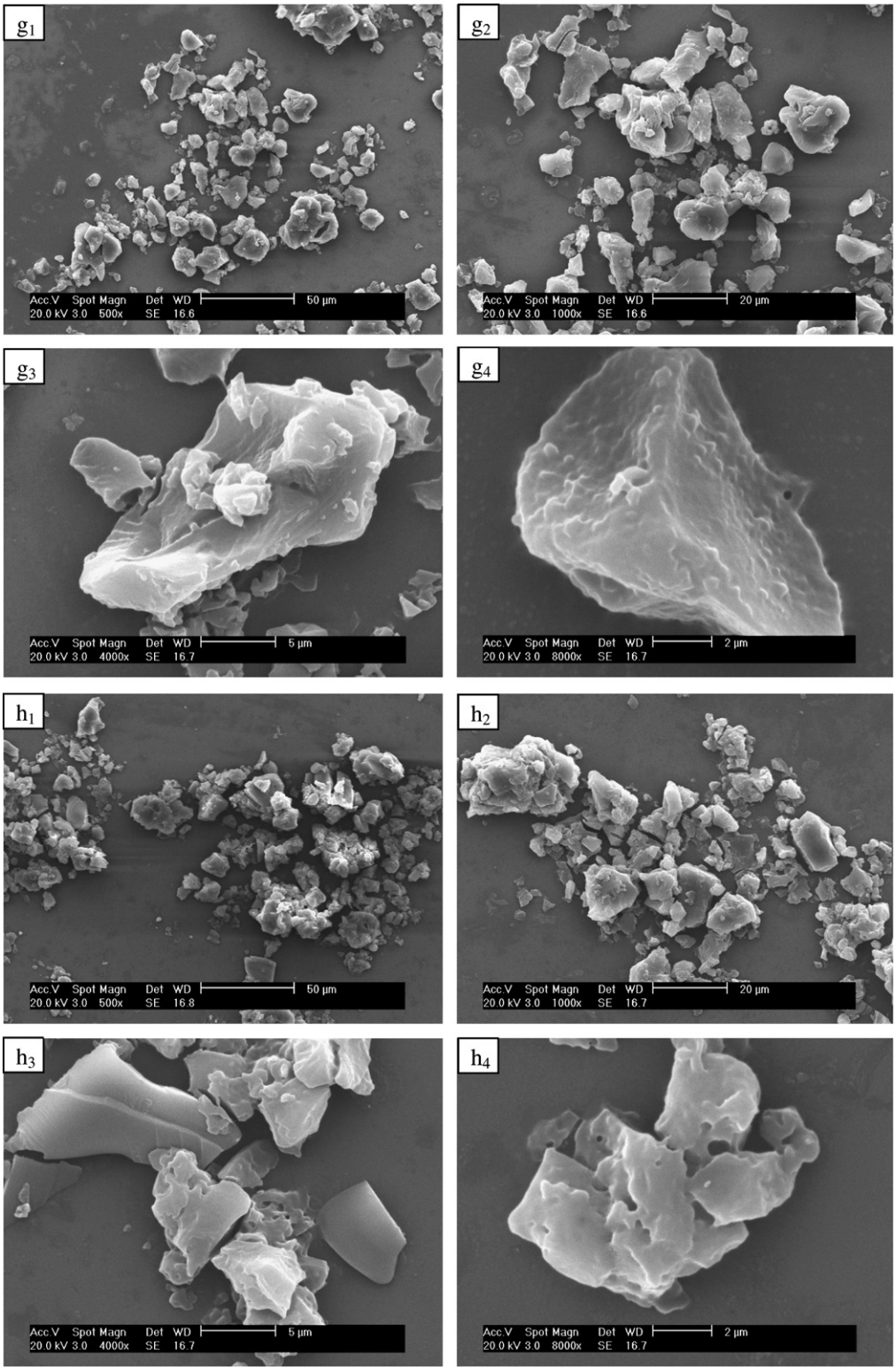

Fig. 2 (*continued*)

And the semi-crystalline growth rings are also hydrolyzed severely (Fig. 2e$_3$). Some fragments with several hundred nanometers from the semi-crystalline growth rings are clearly observed (Fig. 2e$_4$). When the amorphous growth rings are subjected to severe corrosion, the semi-crystalline ones could be clearly seen under SEM (Fig. 2f$_3$ and f$_4$). As the hydrolysis proceeds further, most of the starch granules are destroyed completely which results in the formation of lots of fragments with the size of a few 10s of nanometers (Fig. 2g and h).

3.3. TEM and HR-TEM

After hydrolyzing starch granules for 25 days, aggregates of nanometer-sized fragments could be seen in the TEM images

(Fig. 3a and b). Due to the strong association, the individually well-separated microcrystal is not observable in the TEM images. When observed under HR-TEM, clear lattice fringes with different orientation and the blurry amorphous regions could be seen, as shown in Fig. 3d, e and f. Lattice fringes with a *d*-spacing of 0.304 nm (±0.05 Å) are identified in the images (Fig. 3d). Each of the regions corresponds to an edge-on crystalline lamella which constitutes the crystalline regions of semi-crystalline growth rings (Fig. 2b_4 and d_4). These observations have lead us to conclude that most of the crystalline lamellas are in fact a mixture of multicrystalline phases which could constitute the A- or B-type crystallites in the C-type pea starch granules. This could be interpretated that C-type starch granules are composed of A- and B-type crystallites in which each crystallite is also made up of multicrystalline phase. In addi-

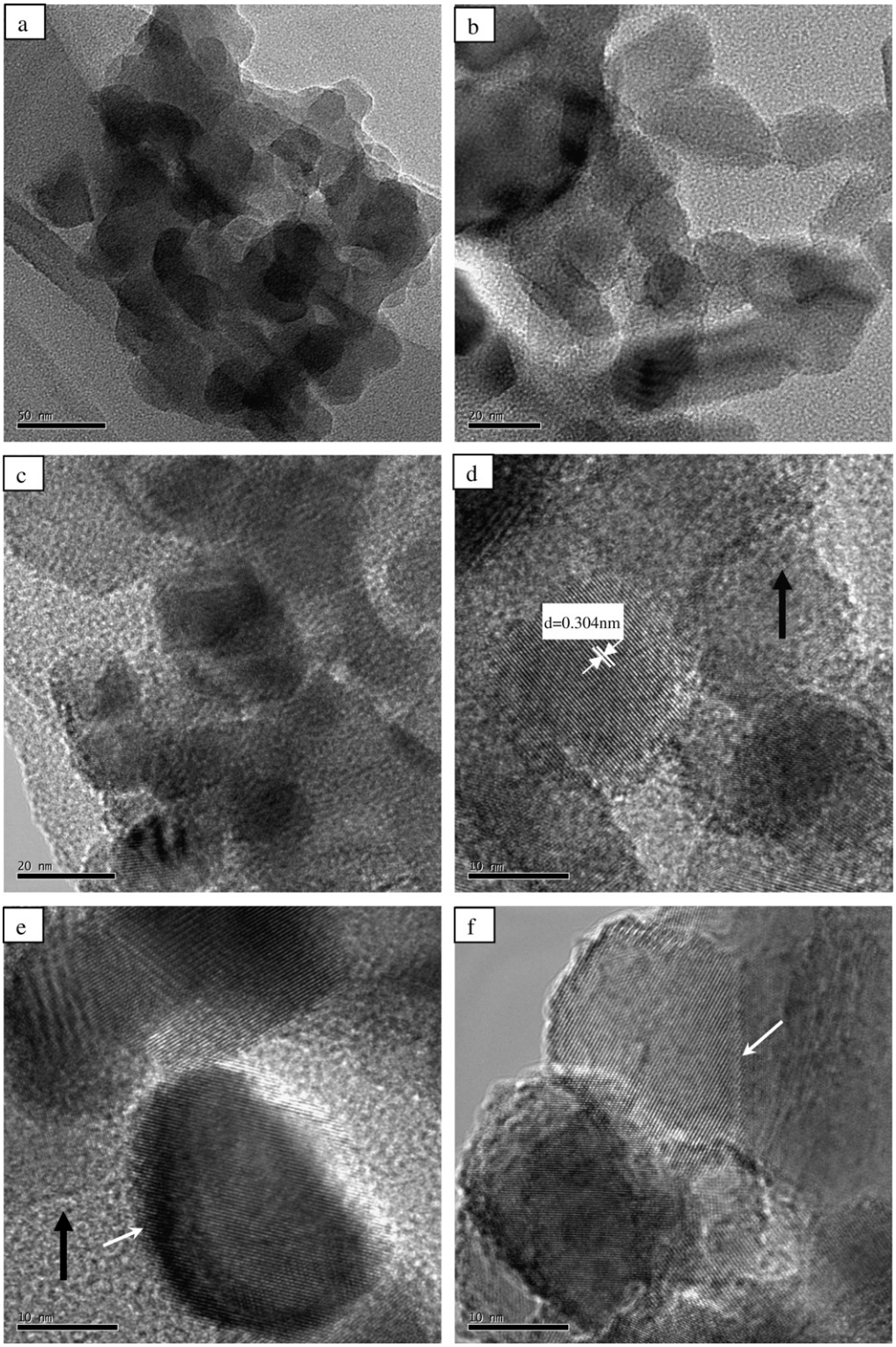

Fig. 3. TEM micrographs of fragments from starch after acid hydrolysis: (a–c) TEM images of fragments from starch granules after 25 days of hydrolysis; (d–f) HR-TEM images of fragments from starch granules after 25 days of hydrolysis.

tion, the clear boundaries (white arrows in Fig. 3e and f) among crystalline and/or amorphous lamellas are also visible. The grain size can be confirmed to be about 20 nm with the length of 20 nm and the thickness of 20 nm. The thickness, in the native granule architecture, corresponds to the length of the amylopectin short chains in double helical conformation. The thickness of the crystalline lamella of pea starch is approximately twice as that of normal crystalline lamella which may be due to the overlap of two parallel crystalline lamellas. From Fig. 2c and d, the amorphous phases (regions indicated by black arrows) could be observed in the HR-REM images which was surrounded by the crystalline phases. These amorphous phases correspond to the amorphous growth rings among the semi-crystalline ones. Our results also suggested that, although the amorphous regions were hydrolyzed faster than the crystalline ones, they were not degraded completely in the duration of hydrolysis.

It is known that the acid preferentially attacks the amorphous regions and then the high crystallinity regions. The amorphous regions being less compact than the crystalline regions results in the quick diffusion of H_3O^+ ions into the amorphous regions. However, the disruption of starch granules and the formation of small crystalline fragments could be detected in the first hydrolysis stage. This proves that the erosion of the semi-crystalline growth rings, although slow, and that of the amorphous areas are concomitant. The corrosion of crystalline lamella starts as soon as the acid can diffuse in the semi-crystalline growth rings of the granules. It appears a continuous process that was illustrated in the SEM images of Fig. 2. Our results suggest that, the amorphous and the crystalline regions are attacked simultaneously by the acid. The amorphous regions in the interior of starch granules are not hydrolyzed until the outer crystalline regions are split open. This is in agreement with TEM results by which amorphous and crystalline phases coexists in the fragments of granules.

3.4. X-ray diffraction

The powder X-ray diffraction patterns for both native and acid-modified starches are shown in Fig. 4. Fig. 4a reveals the main crystalline features of C-type pea starch with a diffraction peak at 6.4° (d = 1.606 nm) that is the indication of B-type starch and a composite series of peaks around 20–21° with a clear hump at 21° (d = 0.491 nm) corresponding to A-type starch. The decrease in intensity of the peak at 6.3° 2θ and the increase in that of the peak at 20.8° 2θ reveals the reduction in the relative proportion of B-type polymorph to A-type polymorph. Additionally, the peaks at around 2θ value of 17.5° and 26.6° become more and more intense with the hydrolysis proceeding. Our results indicate that the B-type allomorph is degraded more rapidly than A-type one. Although B-type allomorph is hydrolyzed faster than A-type one, it still coexists with A-type allomorph in the acid-modified starch granules for 25 days of hydrolysis. The results presented in this study seem not to be in accordance with our previous reports (Wang et al., 2007a, 2007b). The B-type allomorph present in C-type Chinese yam starches could be degraded entirely after 32 days of acid hydrolysis. In the previous experiments, the specimens were equilibrated in the atmosphere (RH = 25%) for one week before the XRD measurements were done. Water content plays an important role in the crystalline nature of starch granules. The B- part of the sample is much susceptible to dehydration as the intra-crystalline water content is more in B-type starch than in A- type starch. The low water content will hamper the crystallization of B-type crystallite. According to this information, it is not difficult to explain that the B-type allomorph was not detected in the XRD patterns after 32 days of hydrolysis. In the present study, the starch samples are placed in an atmosphere (RH = 75%) for one week. The high water content is very beneficial to the crystallization

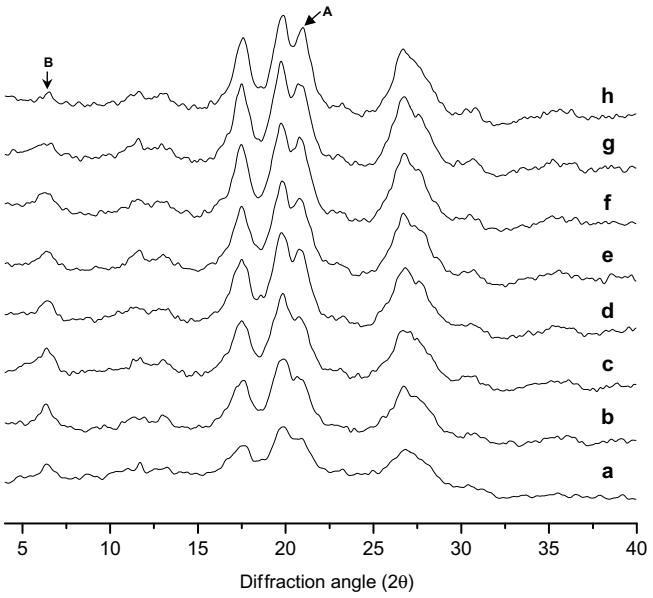

Fig. 4. X-ray powder diffraction spectra of native and acid-modified starches. (a) Native starch; (b) 2 days; (c) 4 days; (d) 8 days; (e) 12 days; (f) 16 days; (g) 20 days; (h) 25 days.

Table 1
X-ray diffraction data of unmodified starch and modified starches

Samples	Crystal-type	Degree of crystallinity (%)
Unmodified starch	C_B	70.3
Acid-thinned 2ds	C_B	72.9
Acid-thinned 4ds	C_B	74.6
Acid-thinned 8ds	C	76.1
Acid-thinned 12ds	C	80.2
Acid-thinned 16ds	C	83.5
Acid-thinned 20ds	C_A	85.6
Acid-thinned 24ds	C_A	88.6

of B-type crystallites. The degree of crystallinity (Table 1) of acid-thinned starches increased with the increase in the hydrolysis time with sharper peaks at 17.7° 2θ and 27.2° 2θ.

4. Conclusions

Granular structure of C-type starch has been studied extensively by many researchers. Bogracheva et al. (1998) used a combination of techniques (including Differential Scanning Calorimetry, X-ray Diffraction, Nuclear Magnetic Resonance) to study the structure and properties of C-type starch from pea seeds. C-type starch granules contained both types of polymorphs, the B-type polymorph is in the centre of the granule and are surrounded by the A-type polymorph. Except for pea (including smooth and wrinkled) starches, Chinese yam starches showing typical C-type pattern are also paid more and more attention by our research group (Wang, Gao, et al., 2006; Wang, Liu, et al., 2006; Wang, Yu, et al., 2006; Wang et al., 2007a, 2007b). Our results showed that amorphous regions basically locate in the core of starch granules or distribute alternately in the crystalline areas. Amorphous areas mainly consist of B-type polymorph while A-type polymorph primarily constitutes the crystalline phase. These conclusions are in agreement with those of Bogracheva et al. SEM observations in this research also demonstrates that amorphous areas are in fact in the centre of C-type pea starch which are surrounded by the crystalline phase.

Although the granular structure of C-type pea starch had already been reported in detail, it is the first time, to our knowledge,

that the semi-crystalline growth rings are observed from the SEM of cracked starch granules. The thickness of the semi-crystalline growth rings is about 200 nm. The severe hydrolysis could produce nanometer-sized starch nanocrystals which could be seen in the HR-TEM images. The thickness of crystalline lamella in the semi-crystalline growth rings is conformed to be approximately 10 nm. This reveals that one semi-crystalline growth rings consists of about twenty pieces of crystalline lamellas. Work is in progress to determine what kind of crystalline structure is major in the crystalline lamella by using selected area electron diffraction pattern. In addition, C-type starches from different origins also need to be studied to find out the differences in the ultrastructure of granules.

References

Bogracheva, T. Y., Morris, V. J., Ring, S. G., & Hedley, C. L. (1998). The granular structure of C-type pea starch and its role in gelatinization. *Biopolymers, 45*, 323–332.

Buléon, A., Colomna, P., Planchot, V., & Ball, S. (1998). Starch granules: Structure and biosynthesis. *International Journal of Biological Macromolecules, 23*, 85–112.

Cairns, P., Bogracheva, T. Y., Ring, S. G., Hedley, C. L., & Morris, V. J. (1997). Determination of the polymorphic composition of smooth pea starch. *Carbohydrate Polymers, 32*, 275–282.

Cameron, R. E., & Donald, A. M. (1992). A small-angle X-ray scattering study of the annealing and gelatinization of starch. *Polymer, 33*, 2628–2635.

Dang, J. M. C., & Copeland, L. (2003). Imaging rice grains using atomic force microscopy. *Journal of Cereal Science, 37*, 165–170.

French, D. (1984). In R. L. Whistler, J. N. BeMiller, & E. F. Paschalls (Eds.), *Starch chemistry and technology* (pp. 183–247). New York, San Diego, CA: Academic Press.

Jane, J.-L., Kasemsuwan, T., Leas, S., Zobel, H., & Robyt, F. (1994). Anthology of starch granule morphology by scanning electron microscopy. *Starch/Staerke, 46*, 121–129.

Jayakody, J., & Hoover, R. (2002). The effect of lintnerization on cereal starch granules. *Food Research International, 35*, 665–680.

Jenkins, P. J., & Donald, A. M. (1997). The effect of acid hydrolysis on native starch granule structure. *Starch/Stearke, 49*, 262–267.

Nara, S., & Komiy, T. (1983). Studied on the relationship between water-saturated state and crystallinity by the diffraction method for moistened potato starch. *Starch, 35*, 407–410.

Oostergetel, G. T., & van Bruggen, E. F. G. (1989). On the origin of a low angle spacing in starch. *Starch/Stearke, 41*, 331–335.

Putaux, J. L., Molina-Boisseau, S., Momaur, T., & Dufresne, A. (2003). Platelet nanocrystals resulting from the disruption of waxy maize starch granules by acid hydrolysis. *Biomacromolecules, 4*, 1198–1202.

Ratnayake, W. S., Hoover, R., Shahidi, F., Perera, C., & Jane, J. (2001). Composition, molecular structure, and physicochemical properties of starches from four field pea (*Pisum sativum* L.) cultivars. *Food Chemistry, 74*, 189–202.

Ridout, M. J., Parker, M. L., Hedley, C. L., Bogracheva, T. Y., & Morris, V. J. (2004a). Atomic force microscopy of pea starch: Origins of image contrast. *Biomacromolecules, 5*, 1519–1527.

Ridout, M. J., Parker, M. L., Hedley, C. L., Bogracheva, T. Y., & Morris, V. J. (2004b). Atomic force microscopy of pea starch granules: Granule architecture of the rug3, rug4, rug5, and the lam mutants. *Carbohydrate Polymers, 65*, 64–74.

Sarko, A., & Wu, H. C. H. (1978). The crystal structures of A-, B- and C-polymorphs of amylose and starch. *Starch/Stearke, 30*, 73–78.

Vandeputte, G. E., & Delcour, J. A. (2004). From sucrose to starch granule to starch physical behavior: A focus on rice starch. *Carbohydrate Polymers, 58*, 245–266.

Vermeylen, R., Goderis, B., Reynaers, H., & Delcour, J. A. (2004). Amylopectin molecular structure reflected in macromolecular organization of granular starch. *Biomarcomolecules, 5*, 1775–1786.

Wang, L. F., & Wang, Y. J. (2001). Structures and physicochemical properties of acid-thinned corn, potato and rice starches. *Starch/Stearke, 53*, 570–576.

Wang, S. J., Gao, W. Y., Liu, H. Y., Chen, H. X., Yu, J. G., & Xiao, P. G. (2006). Studies on the physicochemical, morphological, thermal and crystalline properties of starches separated from different *Dioscorea opposita* cultivars. *Food Chemistry, 99*, 38–44.

Wang, S. J., Liu, H. Y., Gao, W. Y., Chen, H. X., Yu, J. G., & Xiao, P. G. (2006). Characterization of new starches separated from different Chinese yam (*Dioscorea opposita* Thunb.) cultivars. *Food Chemistry, 99*, 30–37.

Wang, S. J., Yu, J. L., Gao, W. Y., Liu, H. Y., & Xiao, P. G. (2006). New starches from Traditional Chinese Medicine (TCM)- Chinese yam (*Dioscorea opposita* Thunb.) cultivars. *Carbohydrate Research, 341*, 289–293.

Wang, S. J., Yu, J. L., Gao, W. Y., Pang, J. P., Liu, H. Y., & Yu, J. G. (2007a). Particle structural changes in native Chinese yam (*Dioscorea opposita* Thunb var. Anguo) starches during acid hydrolysis. *Carbohydrate Polymers, 69*, 286–292 .

Wang, S. J., Yu, J. L., Yu, J. G., Chen, H. X., & Pang, J. P. (2007b). The effect of acid hydrolysis on morphological and crystalline properties of Rhizoma *Dioscorea* starch. *Food Hydrocolloids, 21*, 1217–1222.