Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 69 (2007) 286–292
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Granule structural changes in native Chinese Yam (*Dioscorea opposita* Thunb var. Anguo) starch during acid hydrolysis

Wang Shujun [a,*], Yu Jinglin [b], Gao Wenyuan [a], Pang Jiping [a], Liu Hongyan [c], Yu Jiugao [b]

[a] *School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, China*
[b] *College of Science, Department of Chemistry, Tianjin University, Tianjin 300072, China*
[c] *Henan Agricultural Academy of Sciences, Henan Province 450002, China*

Received 16 July 2006; received in revised form 1 September 2006; accepted 4 October 2006
Available online 28 November 2006

Abstract

Starch extracted from Chinese Yam (*Dioscorea opposita* Thunb var. Angao) was treated with 2.2 mol/L hydrochloric acid for different lengths of time, (2, 4, 8, 16, and 32 days). The recovery yield, morphological, crystalline, and particle size distribution properties were investigated by scanning electron microscope (SEM), wide angle X-ray powder diffractometer (WAXRD), and laser light scattering particle size analysis (LLSPSA). The recovery yield decreased gradually with increasing hydrolysis time. The average particle size of the starch reduced continuously following acid thinning. The particle shape of the acid-thinned starch also changed significantly with acid hydrolysis. The most notable phenomenon was the conversion of thick starch granules (oval or spherical) to thin starch granules (caky or sheet). After acid hydrolysis for 32 days, most of the starch granules had disintegrated with many fragments visible. The crystalline type of the *D. AG* starch changed from C-type to A-type after 8 days of acid hydrolysis. This result was first found in the present study. The crystallinity level increased with increasing hydrolysis time.

Keywords: Starch; Acid-thinned; Morphology; Crystallinity; Granule structure

1. Introduction

Starch is one of the major polysaccharides in plants and is in the form of granules that exists naturally within the plant cells. Starch is semicrystalline in nature with varying levels of crystallinity. The crystallinity is exclusively associated with the amylopectin component, while the amorphous regions mainly represent amylose. Amylose is an α-(1 → 4)-D-glucopyranosyl polymer, with linear or lightly branched structures or a mixture of both. The residues in amylopectin are α-(1 → 4)-D-glucopyranosse units with α-(1 → 6)-linkages at intervals of approximately 20 U, depending on plant sources (Karim, Norziah, & Seow, 2000; Zobel, 1988a, 1988b).

Scanning electron microscope (SEM) has been used to relate granule morphology to starch genotype (Fannon, Hauber, & Bemiller, 1992a). Laser light scattering has been used to characterize granule diameter, based on the assumption that granules are spherical (Wiesenborn, Orr, Casper, & Tacke, 1994). The shape of the starch granule varies with the botanical source of the starch, with approximately oval, elliptic, spherical as well as irregular shapes found. The granule size also depends on the biological origin. X-ray powder diffraction diffractometry has been used to reveal the presence and characteristics of the crystalline structure of the starch granules (Hoover, 2001). Starch can be classified into 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 stronger diffraction peaks at around 15°, 17°, 18°, and 23°. The B-type starch was usually obtained from tuber starches, such as potato

* Corresponding author. Tel.: +86 22 87401895.
E-mail address: edwinwa@hotmail.com (W. Shujun).

0144-8617/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2006.10.005

starch and canna starch. The strongest diffraction peak in the X-ray diffraction pattern appears at 17° 2θ. There are also a few small peaks at 2θ values of 20°, 22°, and 24°. C-type starch is a mixture of both A- and B-types. Examples are smooth-seeded pea starch and various bean starches (Cheetham & Tao, 1998; Elsenhaber & Schulz, 1992; Hizukuri, 1985; Zobel, 1988a).

Starch separated from different cultivars of Chinese Yam (*Dioscorea opposita* Thunb.) has been investigated for physico-chemical (e.g. 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, Yu, Gao, Liu, & Xiao, 2006c; Wang et al., 2006b, 2006a). However, further information on the starch granule structure is very limited. 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 for manufacture of acid-thinned starch involves treating a concentrated starch slurry at a temperature lower than the gelatinization temperature of the starch with mineral acid for a period of time (Wurzburg, 1986). Acid modification could change the morphological properties, crystalline properties, gelatinization properties involving transition temperatures and gelatinization enthalpy, and viscoelastic properties of the starch. So, acid modification of starch could be very helpful in understanding the internal 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).

In this study, the morphological and crystalline changes during the acid hydrolysis were investigated by SEM, XRD, and LLSPSA. The objective of the study was to investigate the internal structure of the starch granules.

2. Materials and methods

2.1. Materials

D. AG starch obtained from dried Rhizoma of *Dioscorea opposita* Thunb. cv. Anguo in our laboratory was used throughout the study.

2.2. Starch isolation and preparation of acid-thinned starch

D. AG starch was isolated from dried Rhizoma of *Dioscorea opposita* cv. Anguo according to the method described in our previous study (Wang et al., 2006c).

Two grams (dry basis) of native *D. AG* starch 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 filtered by a 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 seven. The resulting filter cake was washed 2–4× with acetone again. The resulting acid-thinned starch was dried at room temperature overnight (air stream) and utilized throughout the whole experiment.

2.3. The recovery yield of the starch after acid hydrolysis

The recovery yield of the starch after acid hydrolysis was calculated as the following equation:

$$\text{Recovery Yield } (\%) = W_a/W \times 100\%,$$

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

The acid-thinned starch was collected and heated in the oven at 105 °C for 3 h. The acid-thinned starch was then put in the desiccator and measured after it was cooled.

2.4. Morphological properties

Scanning electron micrographs were obtained with an environmental scanning electron microscope (ESEM, Philips XL-3). Acid-thinned starch samples were suspended in acetone to obtain a 1% suspension. One drop of the starch–acetone suspension was applied on an aluminium 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.

2.5. X-ray diffractometry

X-ray powder diffraction measurements were performed using a Panalytical X'Pert Pro diffractometer (PANalytical, Holand). Each sample of acid-thinned starches was packed tightly in a rectangular glass cell (15 × 10 mm, thickness 0.15 cm). The samples were exposed to the X-ray beam from an X-ray generator running at 40 kV and 40 mA. The scanning regions of the diffraction angle 2θ were 4–35°, which covered most of the significant diffraction peaks of the starch crystallites. The other operating conditions were as follows: $\lambda = 1.78901$, step size, 0.0330°, scan step time, 30.8451 s, divergence slit size, 0.2177°. The *d*-spacings were computed according to Bragg's equations ($n\lambda = 2d\sin\theta$; where $d =$ inter crystalline spacing, $n = 1$, and $\lambda = 1.78901$ Å). Duplicate measurements were made at ambient temperature.

2.6. Determination of the degree of crystallinity

The degree of crystallinity of samples was quantitatively estimated following the method of Nara and Komiy, 1983. A smooth curve which connected peak baselines was computer-plotted on the diffractograms (Fig. 1). The area above the smooth curve was taken as the crystalline fraction, and the lower area between smooth curve and the linear baseline which connected the two points of the

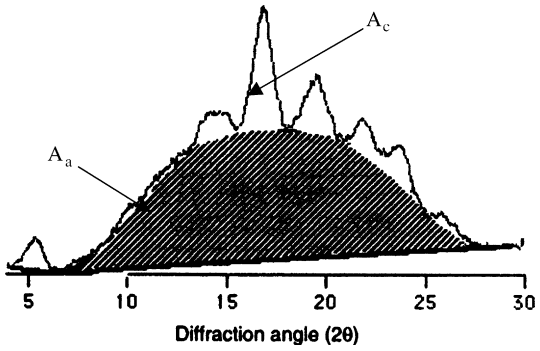

Fig. 1. Calculation of the relative degree of the crystallinity.

intensity 2θ of 30° and 4° in the samples was taken as the amorphous fraction. The upper diffraction peak area and the total diffraction area over the diffraction angle 4–30° 2θ were integrated using Smadchrom software (Morgan and Kennedy Research, Australia). The ratio of upper area to the total diffraction area was taken as the degree of crystallinity.

The equation for 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 refers to the amorphous area on the X-ray diffractogram.

2.7. Particle size analysis

Particle size analysis of acid-thinned *D. AG* starches was done using a laser light scattering particle size analyzer (Mastersizer S, version 2.15, Malvern instruments Ltd., Malvern, UK). The focal length was 100 mm.

3. Results and discussion

3.1. Recovery yield of acid-thinned starch

The recovery yield of the acid-thinned starch was presented in Fig. 2.

The recovery yield of acid-thinned starch decreased gradually with increasing acid hydrolysis time. Hydrolysis was rapid for about the first 8 days and much slower from 8 to 32 days. The initial rapid hydrolysis phase was mainly attributed to the hydrolysis of amorphous material, and the later slower phase to the hydrolysis of the crystalline region. The amorphous areas of the starch granules had a looser structure than the crystalline regions which was easier to attack with the hydrogen ions. Native starches have some flaws on the surface of the granules which could provide channels for the infiltration of hydrogen ions. These hydrogen ions primarily got to the amorphous areas and attacked them (Franco, Cabral, & Tavares, 2002; Wang & Wang, 2001).

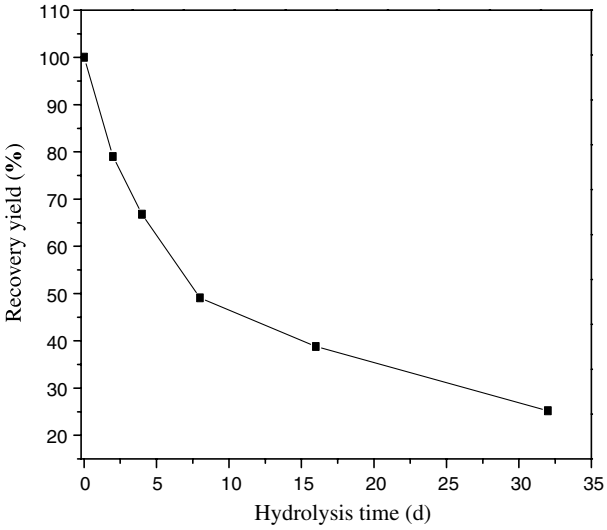

Fig. 2. Recovery yield of starch after acid hydrolysis.

3.2. Scanning electron microscopy (SEM)

Scanning electron photomicrographs of unmodified starch and acid-thinned starches are shown in Fig. 3.

The majority of native starch granules appeared oval and spherical but some of the granules were of irregular shape with size ranging from 8 to 80 μm (Fig. 3a_1 and a_2). No obvious external structural difference between unmodified starch and acid-thinned starch was observed (Fig. 3a and b, respectively). There were some defects on the surface of the acid-thinned starch granules (depicted by arrows in Fig. 3b_1). With increasing hydrolysis time (4 days), some short hairs emerged on the surface of the starch granules which could be due to the release and association of short starch chains during the process of hydrolysis. When the starch granules were subjected to 8 days acid hydrolysis, obvious differences were observed between these starches and those hydrolyzed for shorter time. The surface of the starch granules became less smooth and the size also decreased. The starch granules became much thinner and were bread-like or cake-like in shape. The starch granules started to fracture and lots of cavities appeared on their surfaces when they were hydrolyzed for 16 days. When subjected to 32 days of acid hydrolysis, starch granules showed significant changes in their shape and size. No intact starch granules were observed and all the fragments were conglutinated due to the heavy acid erosion. The above results showed that the core part of the starch granule was more easily attacked by the hydrogen ion. As is well known, the amorphous regions of starch granules was preferentially degraded in the process of acid hydrolysis. So, SEM results indicated that the amorphous regions were mainly located inside of starch granules, while the crystalline areas mainly existed on the outside of the starch granule. That is why the starch granules became thinner during the first stage of acid hydrolysis and fractured at the latter stage.

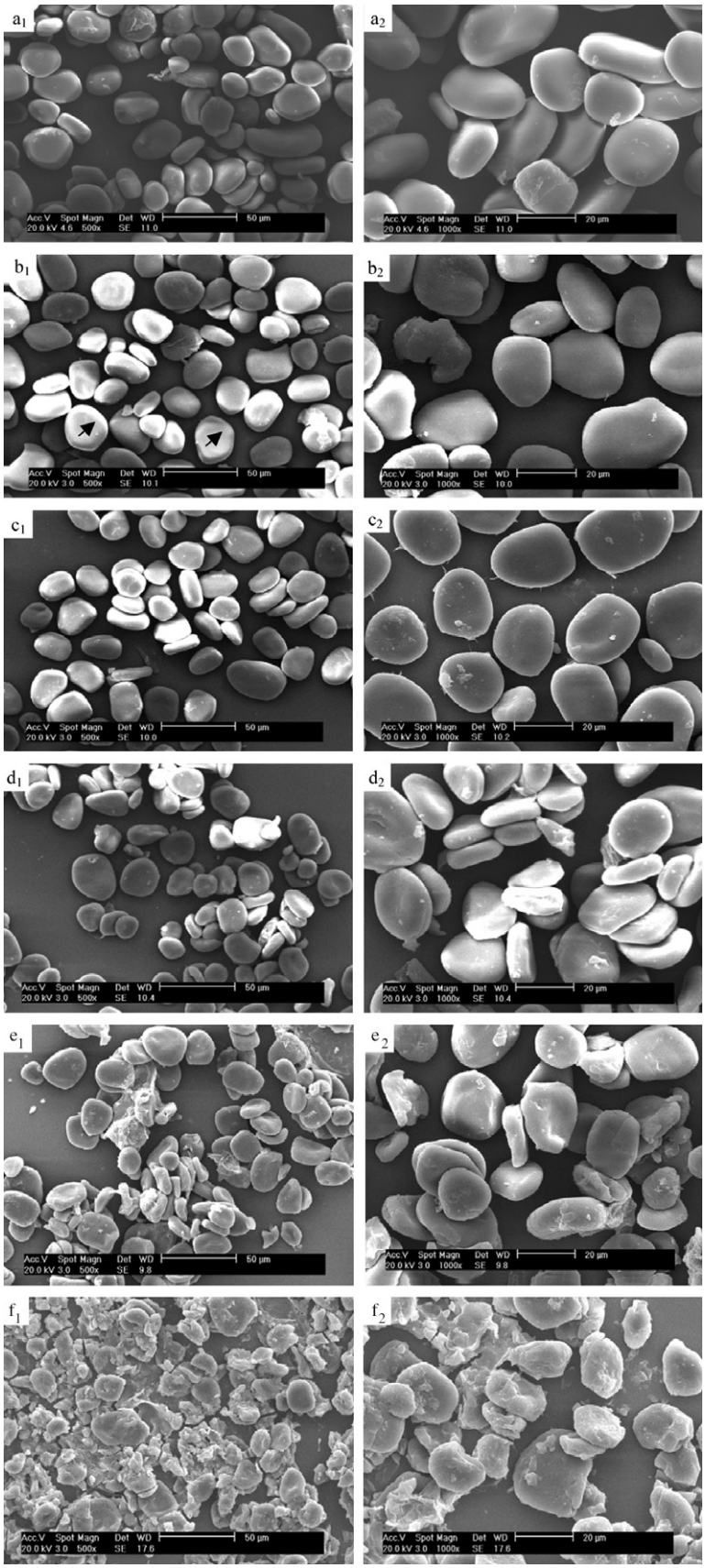

Fig. 3. SEM photographs of unmodified and modified starches a_1, a_2: unmodified starch; b_1, b_2: 2 days; c_1, c_2: 4 days; d_1, d_2: 8 days; e_1, e_2: 16 days; f_1, f_2: 32 days.

3.3. Particle size analysis

Fig. 4 showed the granule size distribution of unmodified starch and acid-thinned starches. The abscissa represented particle diameter (μm) and the volume percent (%) was displayed in *y*-axis. The particle size changes during the hydrolysis are shown in Fig. 5.

3.4. X-ray diffraction

The X-ray diffraction patterns of acid-thinned starches and their native counterpart were shown in Fig. 6. The corresponding X-ray diffraction parameters and crystallinity level calculated from the ratio of diffraction peak area and total diffraction area are given in Table 1.

X-ray diffraction pattern of the *D. AG* starch was typical C-type pattern. The peak at around 2θ value of 6.5° were characteristic of B pattern, while at 27.4° 2θ were indicative of the A pattern. No significant difference was observed between the X-ray diffraction patterns of the unmodified starch and acid-thinned starch for 2 days, except that acid-thinned starch derivatives showed a slightly broader peak at $2\theta = 6.5°$ and sharper peaks at $2\theta = 17.7°$, 20.0°, and 27.0°. The peak at 6.5° 2θ became weaker due to degradation of B-polymorphs present in C-type starch following the acid thinning while the sharper peaks at 17.7°, 20.0°, and 27.0° could be attributed to the increased crystallinity with acid thinning. With increasing acid hydrolysis time, the crystalline type gradually changed from C-type to A-type. After 4 days of hydrolysis, the peak at around 2θ value of 6.5° became much weaker than that of the native starch. The peak at 20.0° 2θ was split into two small peaks at 20.0° 2θ and 21.0° 2θ which indicated that the A-type polymorphs predominated in the acid-thinned starch. The

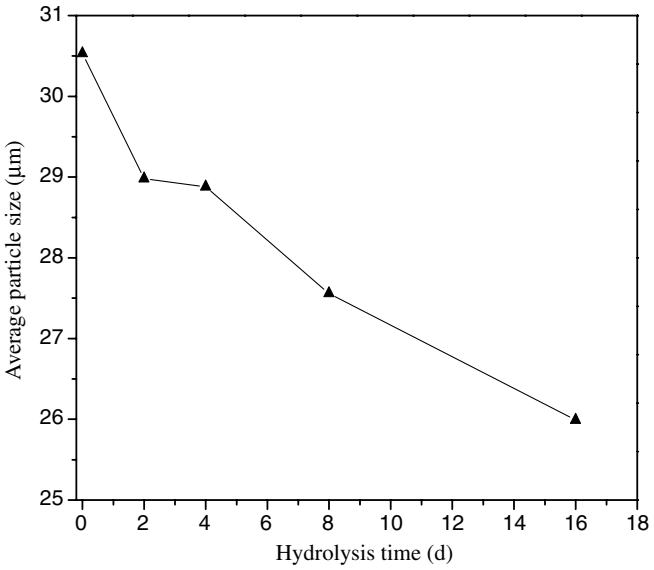

Fig. 5. Effect of acid time on the particle size of modified and unmodified starch.

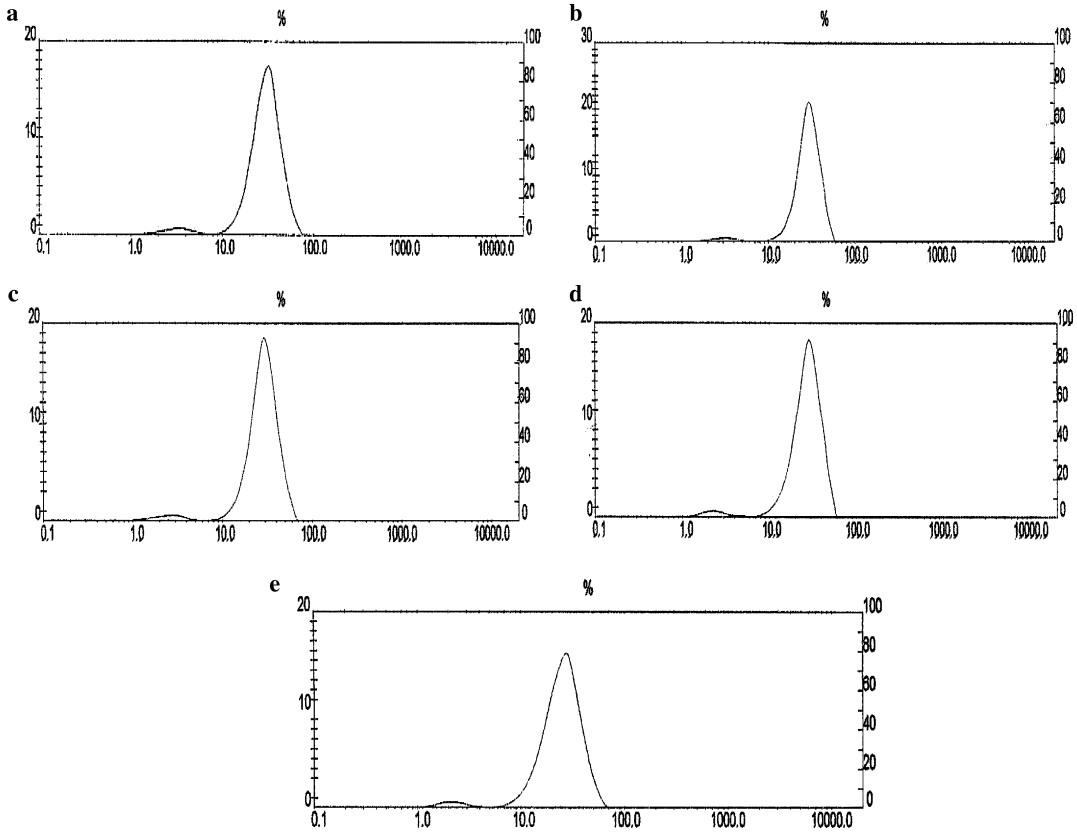

Fig. 4. Particle size distribution of unmodified starch and modified starches a: unmodified starch; b: 2 days; c: 4 days; d: 8 days; e: 16 days.

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

Samples	Diffraction peaks at 2θ values (° angle)					Degree of crystallinity (%)
	6°	18°	20°	21°	27°	
Unmodified starch	6.60	17.65	20.01	–	26.91	43.1
Acid-thinned 2 days	6.49	17.58	19.76	–	27.00	59.8
Acid-thinned 4 days	6.49	17.53	19.91	20.98	26.85	63.6
Acid-thinned 8 days	–	17.38	19.71	20.88	26.40	66.1
Acid-thinned 16 days	–	17.58	19.86	20.98	26.60	69.3
Acid-thinned 32 days	–	17.55	19.88	20.99	26.56	70.7

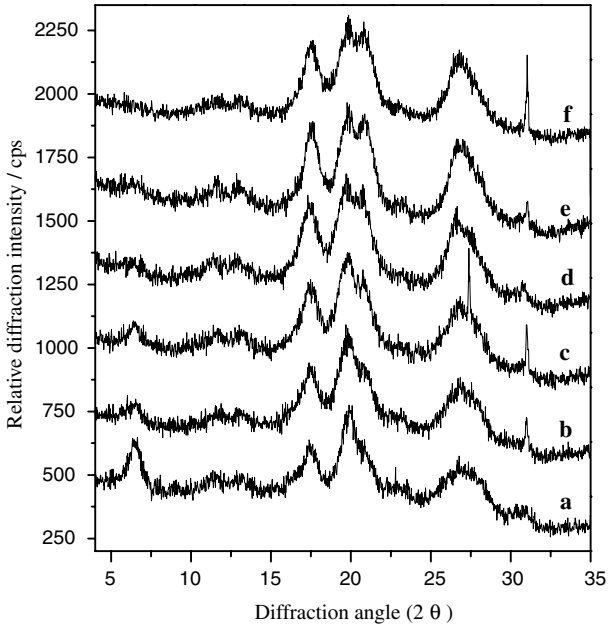

Fig. 6. X-ray diffraction spectraof *D. AG* starches after acid degradation a: 0 days; b: 2 days; c: 4 days, d: 8 days; e: 16 days; f: 32 days.

peak at 6.5° 2θ disappeared and the peak 20.0° 2θ was split into two small peaks at 20.0° 2θ and 21.0° 2θ since the starch was hydrolyzed for 8 days. This result was not consistent with that of other acid-thinned starches which exhibited the same crystalline type as the unmodified starch (Atichokudomchai & Varavinit, 2003; Lawal et al., 2005; Olayide, 2004; Wang, Truong, & Wang, 2003). This result revealed that the B-polymorphs present in the C-type starch was first degraded during the process of acid hydrolysis. Generally, amorphous areas were first hydrolyzed followed by the crystalline regions. That is to say, B-polymorphs contained in C-type starch were associated with the majority of the amorphous regions. The B-polymorphs existed in the centre of C-type starch granules which were surrounded by the A-polymorphs. The crystalline granular structure of Chinese yam is similar to that of pea starch granules (Elsenhaber & Schulz, 1988).

The degree of crystallinity of acid-thinned starches increased with increasing the hydrolysis time. The amorphous region of starch granules was firstly hydrolyzed which resulted in a high content of crystalline regions. It has been suggested that cleavage of starches chains in the amorphous regions allowed extensive reordering of the chain segments to give a more crystalline structure with a sharper X-ray pattern (Wang & Wang, 2001).

4. Conclusion

The particle morphology and crystalline structure of starch separated from *D. AG* changed significantly during the process of acid hydrolysis. The recovery yield of the acid-thinned starch decreased with increasing hydrolysis time. The amorphous regions were mainly located in the centre of starch granules, while the crystalline areas mainly existed outside of the starch granules. The crystalline type of starch transformed from C-type to A-type with hydrolysis. B-polymorphs contained in C-type starch mainly constituted the amorphous regions while the crystalline areas were primarily resulted from the A-polymorphs. The B-polymorphs existed in the centre of C-type starch granules which were surrounded by the A-polymorphs.

References

Atichokudomchai, N., & Varavinit, S. (2003). Characterization and utilization of acid-modified cross-linked tapioca starch in pharmaceutical tablets. *Carbohydrate Polymers, 53*, 263–270.

Cheetham, N. W. H., & Tao, L. P. (1998). Variation in crystalline type with amylose content in maize starch granules: an X-ray powder diffraction study. *Carbohydrate Polymer, 36*, 277–284.

Elsenhaber, F., & Schulz, W. (1992). Monte carlo simulation of the hydration shell of double-helical amylase: a left-handed antiparallel double helix fits best into liquid water structure. *Biopolymers, 32*, 1643–1664.

Fannon, J. E., Hauber, R. J., & Bemiller, J. N. (1992a). Surface pores of starch granules. *Cereal Chemistry, 69*, 284–288.

Franco, C. M. L., Cabral, R. A. F., & Tavares, D. Q. (2002). Structural and physicochemical properties of Lintnerized native and sour cassava starches. *Starch, 54*, 469–475.

Hizukuri, S. (1985). Relationship between the distribution of the chain length of amylopect in and the crystalline structure of starch granules. *Carbohydrate Polymers, 141*, 295–306.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate polymer, 45*, 253–267.

Kang, K. J., Kim, S., Lee, S. K., & Kim, S. K. (1997). Relationship between molecular structure of acid-hydrolyzed rice starch and retrogradation. *Korean Journal of Food Science and Technology, 29*, 876–881.

Karim, A. A., Norziah, M. H., & Seow, C. C. (2000). Methods for the study of starch retrogradation. *Food Chemistry, 71*, 9–36.

Kim, R. E., & Ahn, S. Y. (1996). Gelling properties of acid-modified red bean starch gels. *Agriculture Chemistry and Biotechnology, 39*, 49–53.

Lawal, O. S., Adebowale, K. O., Ogunsanwo, B. M., Barba, L. L., & Ilo, N. S. (2005). Oxidized and acid thinned starch derivatives of hybrid maize: functional characteristics, wide-angle X-ray diffractometry and thermal properties. *International Journal of Biological Macromolecules, 35*, 71–79.

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.

Olayide, S. L. (2004). Composition, physicochemical properties and retrogradation characteristics of native, oxidised, acetylated and acid-thinned new cocoyam (*Xanthosoma sagittifolium*) starch. *Food Chemistry, 87*, 205–218.

Rohwer, R. G., & Klem, R. E. (1984). Acid-modified starch: production and used. In J. N. Bemiller & E. F. Paschall (Eds.), *Starch: Chemistry and technology* (pp. 529–541). Orlando, FL: Academic press.

Shi, Y.-C., & Seib, P. A. (1992). The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydrate Research, 227*, 131–145.

Virtanen, T., Autio, K., Suortti, T., & Poutanen, K. (1993). Heat-induced changes in native and acid-modified oat starch paste. *Journal of Cereal Science, 17*, 137–145.

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

Wang, S. J., Gao, W. Y., Liu, H. Y., Chen, H. X., Yu, J. G., & Xiao, P. G. (2006a). 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. (2006b). 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. (2006c). New starches from traditional Chinese medicine (TCM)-Chinese yam (*Dioscorea opposita* Thunb) cultivars. *Carbohydrate Research, 341*, 289–293.

Wang, Y. J., Truong, V. D., & Wang, L. F. (2003). Structures and rheological properties of corn starch as affected by acid hydrolysis. *Carbohydrate Polymers, 52*, 327–333.

Wiesenborn, D. P., Orr, P. H., Casper, H. H., & Tacke, B. K. (1994). Potato starch paste behavior as related to some physical/chemical properties. *Journal of food Science, 59*, 644–648.

Wurzburg, O. B. (1986). Converted starch. In O. B. Wurzburg (Ed.), *Modified starches: Properties and uses* (pp. 17–40). Boca Raton, FL: CRC Press.

Zobel, H. F. (1988a). Starch crystal transmissions and their industrial importance. *Starch, 40*, 1–7.

Zobel, H. F. (1988b). Molecules to granules-a comprehensive starch review. *Starch, 40*, 44–50.