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# Susceptibility of annealed starches to hydrolysis by α-amylase and glucoamylase

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**Abstract**

The objective of this work was to determine if annealing altered the susceptibility of different starches to enzyme hydrolysis. Five commercial starches, including waxy corn, common corn, Hylon V, Hylon VII, and potato, were annealed by a multiple-step process, and their susceptibility to α-amylase and glucoamylase and the physicochemical properties of the hydrolyzed native and annealed starches were determined. During 36 h of enzyme hydrolysis, significant differences were noted between annealed starch and its native counterpart in the extent of α-amylolysis for Hylon V, Hylon VII, and potato, and in the extent of glucoamylolysis for potato. Waxy and common corn starches were hydrolyzed to a greater degree by both enzymes when compared with the other starches. The apparent amylose content of both native and annealed starches decreased during α-amylolysis for all starches, but increased for Hylon V, VII, and potato starches during glucoamylolysis. Most native and annealed starches exhibited comparable or increased peak gelatinization temperatures and comparable or decreased gelatinization enthalpy on hydrolysis with the exception of annealed potato starch, which showed a significant decrease in peak gelatinization temperature on hydrolysis. Annealed starches displayed significant higher peak gelatinization temperatures than their native counterparts. The intensity of main X-ray diffraction peaks of all starches decreased upon hydrolysis, and the changes were more evident for glucoamylase-hydrolyzed starches. The annealing process allowed for a greater accessibility of both enzymes to the amorphous as well as the crystalline regions to effect significant changes in gelatinization properties during enzyme hydrolysis.


*Keywords:* Starch; Annealing; Enzyme hydrolysis; α-Amylase; Glucoamylase

## 1. Introduction

Annealing is the process of incubating starch in excess water at a temperature above the glass transition temperature but below the gelatinization temperature of the starch (Yost & Hoseney, 1986). Under the annealing conditions, the amorphous starch molecules become mobile and reorganize to form an enhanced crystalline structure, resulting in an increase in starch overall crystallinity (Jacobs, Eerlingen, Rouseu, Colonna, & Delcour, 1998; Nakazawa & Wang, 2003; Waduge, Hoover, Vasanthan, Gao, & Li, 2006; Yost & Hoseney, 1986). Annealing, however, does not change the X-ray diffraction pattern (Stute, 1992). Although the molecular mechanism of starch annealing is still not well elucidated, several explanations have been proposed, such as the twisting of unordered free ends of amylopectin A-chains (Kiseleva et al., 2005), an improved alignment of amylopectin double helices within the crystalline lamellae (Kiseleva et al., 2005), and an enhanced glassy structure of the amorphous lamellae (Tester & Morrison, 1990). Furthermore, annealing affects physiochemical properties such as increased gelatinization temperatures and narrowed gelatinization temperature ranges with increased or unchanged enthalpy values (Hoover & Vasanthan, 1994; Knutson, 1990; Kohyama & Sasaki, 2006; Stute, 1992).

The susceptibility of native starch granules to amylolytic enzymes has been studied (Gallant, Bouchet,

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Buleon, & Perez, 1992; Lauro, Suortti, Autio, Linko, & Poutanen, 1993; Leach & Schoch, 1961; Zhou, Hoover, & Liu, 2004). A biphasic trend has been observed with an initial rapid hydrolysis of the amorphous regions (Franco, Ciacco, & Tavares, 1988; Gallant et al., 1992; Hoover & Vasanthan, 1994; Zhou et al., 2004) followed by a decreased hydrolysis. Some researchers proposed that the amorphous and crystalline regions were hydrolyzed at a similar ratio (Lauro, Forssell, Suortti, Hulleman, & Poutanen, 1999; Leach & Schoch, 1961; Lin et al., 2006).

Starches of different sources display considerable differences in their susceptibility to enzyme action. Potato starch with B-type X-ray diffraction pattern is more resistant to amylolysis than are cereal starches with A-type pattern. Kimura and Robyt (1996) proposed that potato starch had a higher degree of crystallinity than the one measured by X-ray diffractometry. They proposed that the double helical chains in potato starch were formed by both amylose and amylopectin but not associated with each other; therefore the measured crystallinity of potato starch is relatively low. Jane, Wong, and McPherson (1997) postulated that the difference in amylolysis among different crystalline types arrived from variation in the location of their amylopectin branch points. The presence of more A-chains (DP 6–12) and branch linkages in the crystalline lamellae of A-type starches produced 'weak' points that were more susceptible to enzyme hydrolysis. In B-type starches more branch points are found in the amorphous region and thereby provide a more superior crystalline structure that is resistant to hydrolysis. Gallant, Bouchet, and Baldwin (1997) proposed that α-amylolysis was affected by the size and arrangement of starch molecules in the amorphous and crystalline lamellae and their interactions with non-starch components. Recently, Zhou et al. (2004) proposed that the formation of crystalline regions from hydrolyzed amylose chains during hydrolysis could also hinder the accessibility of α-amylase to glucosidic bonds. Some researchers proposed that the resistance of potato starch (B-type) to enzyme hydrolysis may be attributed to its larger blocklets arranged near the surface compared with smaller blocklets in A-type starches (Baldwin, Adler, Davies, & Melia, 1998; Gallant et al., 1992, 1997; Lin et al., 2006).

Recently, Nakazawa and Wang (2003, 2004) demonstrated that in addition to perfecting the crystalline structure, annealing also created void, porous structure that allowed for more rapid hydrolysis by acid. However, the enzyme susceptibility of native annealed starches has been limited reported. The objective of this study was to investigate the effect of annealing on the susceptibility of starches to the degradation by α-amylase, an endo-enzyme, and glucoamylase, an exo-enzyme. Starches of different sources and amylose contents were included to better understand their impacts on enzyme hydrolysis after annealing.

## 2. Materials and methods

### 2.1. Materials

Native waxy corn, common corn, Hylon V (50% amylose), and Hylon VII (70% amylose) starches were kindly donated by National Starch and Chemical Company (Bridgewater, NJ). Potato starch was obtained from Avebe America Inc. (Princeton, NJ). α-Amylase and glucoamylase were purchased from Sigma–Aldrich (St. Louis, MO) and used as received without further treatment. One unit of α-amylase (A-7595; *Bacillus amyloliquefaciens,* 288,000 U/mL) will dextrinize 5.26 g starch (db) per hour under standard conditions. One unit of glucoamylase (A-3042; *Aspergillus niger*, 11,500 U/mL) will produce 1.0 mg of glucose from starch in 3 min at pH 4.5 and 55 °C.

### 2.2. Preparation of annealed starch

Starches were annealed by a multiple-step process as described in Nakazawa and Wang (2003). A multi-step annealing process was employed because it has been shown to produce higher gelatinization temperatures and more perfect reorganization than either one or two-step processes (Knutson, 1990). Starch (100 g, db) and distilled water (300 mL) were placed in a 500-mL beaker, covered with aluminum foil, and incubated at 40 °C and then 5 °C higher intervals until 55, 55, 60, 60, and 55 °C for waxy corn, common corn, Hylon V, Hylon VII, and potato, respectively. The highest annealing temperature for each starch was selected according to the results by Nakazawa and Wang (2003). Starch was annealed at each annealing temperature for 24 h. After the annealing treatment, starch was filtered through a Whatman No. 4 filter paper and dried at room temperature.

### 2.3. Enzymatic hydrolysis of starch granules

A slurry containing 12.5 g starch (db), native or annealed, and 37.5 mL buffer was incubated at 50 °C with constant shaking at 145 rpm in a reciprocating shaker (Boekel Scientific, Feasterville, PA). The buffer in α-amylolysis was 20 mM phosphate buffer at pH 6.9, whereas that of the glucoamylolysis was 20 mM acetate buffer at pH 4.5. Hydrolysis was initiated by the addition of 200 U enzyme/g dry starch to the slurry. Aliquots of 5 mL were taken after 1 h and frequently thereafter until 36 h. At least 4 slurry samples were prepared for each starch type for the enzyme hydrolysis in order to collect duplicate samples during the course of 36 h. The aliquots were centrifuged at 1520*g* for 15 min, and the supernatant was immediately determined for soluble sugars content by using the phenol-sulfuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The starch was dried in a 40 °C oven for 48 h, powdered, and sieved through a US Standard Sieve #100 with a sieve opening of 150 μm. Two hydrolyzed samples were prepared from each starch type for each enzyme.

$$\text{Degree of hydrolysis (\%)} = \frac{\text{Solublized sugars produced by enzyme hydrolysis}}{\text{Total starch weight (d.b.)}} \times 100$$

### 2.4. Apparent amylose content

The amylose content of enzyme-treated native and annealed starches was calorimetrically determined according to the method of Juliano et al. (1981). Potato amylose (Sigma A-0512) and waxy rice starch were used to construct the standard curve.

### 2.5. Starch morphology

Scanning electron micrographs of enzyme-treated native and annealed starches were taken with a Philips XL-30 scanning electron microscope (Philips Electron Optics, Eindhoven, Netherlands) at an accelerating voltage of 6.0 kV. Starch granules were sprinkled onto double-backed cellophane tape attached to a stub before coating with gold-palladium.

### 2.6. Thermal properties

Thermal properties were assessed by a Perkin-Elmer Pyris-1 differential scanning calorimetry (DSC, Perkin-Elmer Co., Norwalk, CT). The instrument was calibrated with indium and an empty pan was used for reference. Starch (~4.0 mg, d.b.) was weighed into an aluminum DSC pan and then moistened with 8.0 μL of deionized water using a microsyringe. The pan was hermetically sealed and allowed to stand for 1 h prior to analysis. The sample was scanned from 25 °C to 130 °C at a rate of 10 °C/min. The onset ($T_o$), peak ($T_p$) and conclusion ($T_c$) gelatinization temperature and enthalpy ($\Delta H$) were automatically computed. Because of the thermograms of Hylon V and VII were not symmetrical and difficult to precisely determine by using the software, gelatinization temperatures were manually determined, and a planimeter (Model L-30, Los Angeles Scientific Instrument Co., Inc., Los Angeles, CA) was used to determine $\Delta H$ by measuring the area under the transition peak.

### 2.7. X-ray diffraction

X-ray diffraction patterns of starches were obtained by a Phillips Analytical diffractometer (Philips, Almelo, Netherlands) with a copper anode X-ray tube. The diffractometer was operated at 27 mA and 50 kV, and the reflection angle ($2\theta$) was from 5° to 45° at 0.1° step size with a count time of 2 s. A 100% relative humidity chamber was used to equilibrate starch samples for 24 h prior to scanning. The total area and amorphous area were measured with a planimeter. A straight line connecting the two points at 5° and 45° was drawn and considered as the baseline. All the base points of each diffraction peak from 5° and 45° was drawn as a border line separating the crystalline and amorphous regions. The area above the border line was the crystalline region, and the area under the line was the amorphous region. The relative crystallinity (%) was calculated as follows.

$$\text{Relative crystallinity (\%)} = \frac{\text{Total area} - \text{Amorphous area}}{\text{Total area}} \times 100$$

### 2.8. Experimental design

A $5 \times 2 \times 2$ completely randomized design (CRD) (5 starch types, with and without annealing treatment, and two enzymes) was used. Each combination and subsequent analysis was performed in duplicate. Data were statistically analyzed by the JMP program (Version 6, SAS Software Institute, Inc. Cary, NC). Analysis of variance (ANOVA) was used to detect significant differences and Student's $t$ test ($p < .05$) was used to identify significantly different means. All significant differences were reported at the 95% confidence interval.

## 3. Results and discussion

### 3.1. Enzymatic hydrolysis of starch granules

Two different types of amylolytic enzymes, α-amylase, an endo-enzyme, and glucoamylase, an exo-enzyme, were employed in this study to understand if annealing would affect their degradation rates and extents differently. Five starches were studied to relate their changes in physicochemical properties to starch type upon hydrolysis.

Table 1
Degree of hydrolysis (%) of native and annealed starches by α-amylase and glucoamylase*

| | Duration (h) | α-Amylolysis | | Glucoamylolysis | |
|---|---|---|---|---|---|
| | | Native | Annealed | Native | Annealed |
| Waxy corn | 5 | 13.6$^{c}$ | 18.6$^{b}$ | 39.7$^{a}$ | 44.8$^{a}$ |
| | 15 | 21.1$^{c}$ | 22.5$^{b}$ | 56.2$^{a}$ | 59.2$^{a}$ |
| | 36 | 30.0$^{b}$ | 30.6$^{b}$ | 66.7$^{a}$ | 67.6$^{a}$ |
| Common corn | 5 | 12.5$^{c}$ | 18.7$^{b}$ | 25.6$^{a}$ | 29.0$^{a}$ |
| | 15 | 21.6$^{b}$ | 24.9$^{b}$ | 39.0$^{a}$ | 42.9$^{a}$ |
| | 36 | 26.9$^{d}$ | 27.7$^{c}$ | 48.7$^{b}$ | 52.6$^{a}$ |
| Hylon V | 5 | 8.2$^{b}$ | 13.2$^{a}$ | 11.3$^{ab}$ | 11.9$^{ab}$ |
| | 15 | 12.0$^{c}$ | 15.3$^{b}$ | 20.9$^{a}$ | 21.2$^{a}$ |
| | 36 | 13.6$^{c}$ | 16.0$^{b}$ | 26.3$^{a}$ | 26.3$^{a}$ |
| Hylon VII | 5 | 5.9$^{b}$ | 8.7$^{a}$ | 7.2$^{a}$ | 8.5$^{a}$ |
| | 15 | 9.3$^{c}$ | 11.9$^{b}$ | 15.3$^{a}$ | 15.8$^{a}$ |
| | 36 | 11.1$^{d}$ | 13.3$^{c}$ | 21.1$^{a}$ | 20.2$^{b}$ |
| Potato | 5 | 3.3$^{b}$ | 10.2$^{a}$ | 1.8$^{b}$ | 11.2$^{a}$ |
| | 15 | 7.7$^{b}$ | 14.2$^{a}$ | 4.7$^{c}$ | 14.1$^{a}$ |
| | 36 | 12.2$^{b}$ | 15.9$^{a}$ | 11.3$^{b}$ | 15.6$^{a}$ |

* Means of two measurements followed by a common letter in the same row are not significantly different ($p < .05$).

Waxy corn starch is a cereal starch that has ~100% amylopectin and A-type X-ray diffraction pattern. Corn starch is a cereal starch (A-type) that has ~27% amylose and ~73% amylopectin. Hylon V is a cereal starch (B-type) that has ~50% amylose and ~50% amylopectin. Hylon VII is a cereal starch (B-type) that has ~70% amylose and ~30% amylopectin. Potato starch is a tuber starch (B-type) that has ~20% amylose and 80% amylopectin. Selected results of enzyme hydrolysis of native and annealed starches are listed in Table 1, and all results are depicted in Fig. 1. The results showed that the hydrolysis kinetics of native and annealed starch granules was affected by annealing treatment and starch type.

The extent of hydrolysis by α-amylase followed the order: waxy corn ~ common corn > Hylon V ~ Hylon VII ~ potato for both native and annealed starches ($p < .05$). The hydrolysis by glucoamylase followed the order of waxy corn > common corn > Hylon V ~ Hylon VII > potato for native starches, and the order of waxy corn > common corn > Hylon V ~ Hylon VII ~ potato for annealed starches ($p < .05$). A much faster hydrolysis at the initial stage was observed for most annealed starches when compared with their native ones. During the course of 36-h hydrolysis, there were significant differences between the annealed starch and its native counterpart in the extent of α-amylolysis for Hylon V, Hylon VII, and potato, and in the extent of glucoamylolysis for potato. Native potato displayed a linear gradual increase in hydrolysis with time, whereas annealed potato exhibited a rapid increase at the initial stage and then reached a plateau of ~16% conversion.

Kimura and Robyt (1995) and Yook and Robyt (2002) reported a similar trend with native starches by glucoamylase and α-amylase, respectively. Waxy maize starch was found to be most susceptible to glucoamylase, followed by an intermediate group of barley, maize, and tapioca starch, and then the least susceptible group of potato, amylomaize-7 and shoti starches (Kimura & Robyt, 1995). The extent of conversion by both porcine pancreatic α-amylase and *B. amyloliquefaciens* α-amylase followed the order of waxy maize ~ maize > amylomaize-

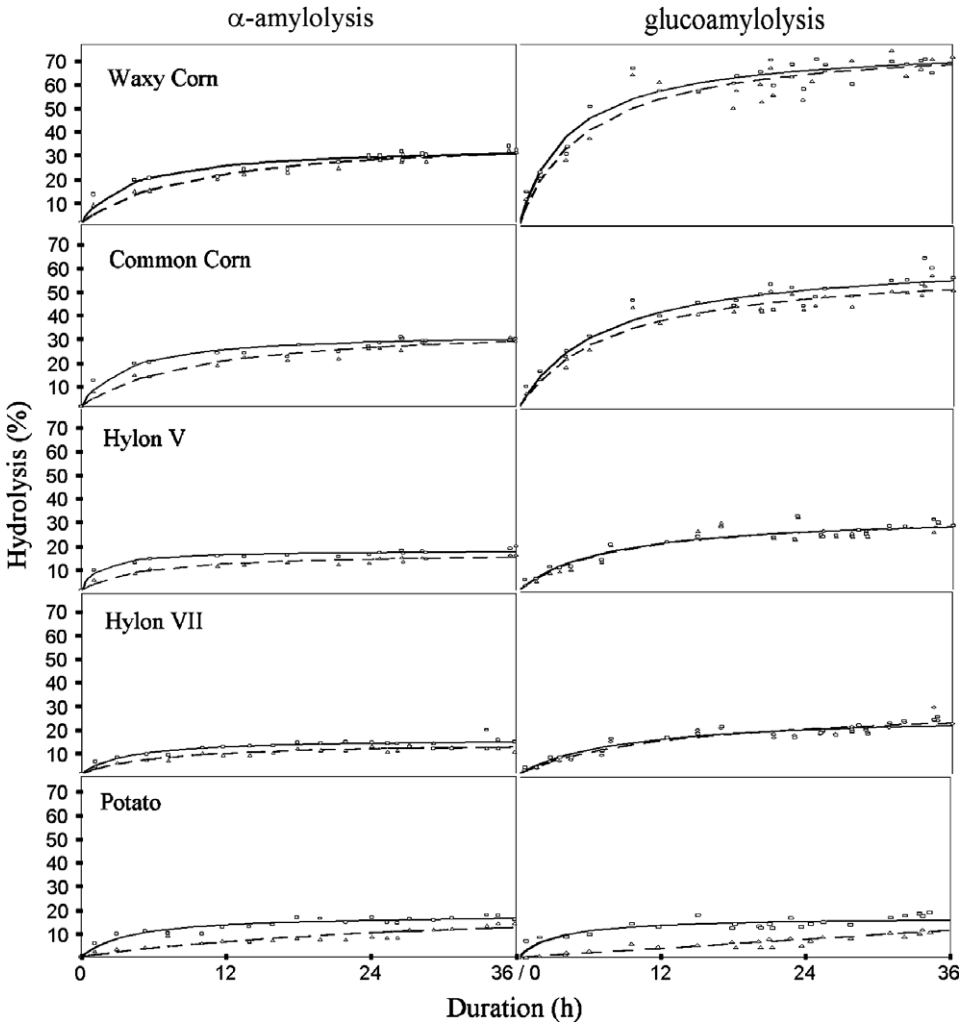


Fig. 1. Percent hydrolysis by α-amylase or glucoamylase of native (–△–) and annealed (—□—) waxy corn, common corn, Hylon V, Hylon VII, and potato starches over 36 h.

7 > potato (Yook & Robyt, 2002). The high resistance to amylolysis of potato starch was ascribed to its high percentage of double-helical chains formed by amylose and amylopectin, whereas that of amylomaize-7 was attributed to a high percentage of inter-double-helical chain association (Kimura & Robyt, 1995). The high amylose content probably hindered the enzyme action by interacting among them and/or with amylopectin during hydrolysis.

Wang, Powell, and Oates (1997) studied the annealing effect on the hydrolysis of sago starch granules by a mixture of α-amylase and glucoamylase. They reported that annealed sago starch was more susceptible to enzyme hydrolysis, which was proposed to result from disruption of hydrogen bonding between the amorphous and crystalline regions and a slight expansion of the amorphous region after annealing. However, it was later reported that annealing did not change the crystalline and amorphous lamellae repeat distance in wheat and potato starches (Jacobs et al., 1998). Nakazawa and Wang (2003) observed more rapid acid hydrolysis of annealed starches relative to their native counterparts, and proposed the formation of more porous structures as a result of annealing. These porous structures might or might not enhance enzyme hydrolysis, which possibly depends on starch type and enzyme type.

The reordering from annealing did not change α-amylolysis nor glucoamylolysis of waxy corn and common corn, but increased α-amylolysis of Hylon V, VII, and potato and glucoamylolysis of potato. The more compact A-type structure might not allow for sufficient change in terms of the porous structure from annealing (Nakazawa & Wang, 2003) to promote enzyme hydrolysis. On the other hand, potato starch exhibited the most increase in degree of enzyme hydrolysis after annealing, presumably due to its B-type less compact structure. Annealed Hylon V and VII exhibited a similar extent of glucoamylolysis but increased α-amylolysis when compared with their native ones. It is known that the action of *B. amyloliquefaciens* α-amylase involves multiple attacks along a binding site having nine D-glucosyl residues (Robyt & French, 1963), whereas glucoamylase requires a starch-binding domain that is distinct from the starch-hydrolyzing domain (Stoffer, Frandsen, Busk, & Schneider, 1993; Svensson, Larsen, Svendsen, & Boel, 1983). The different modes of action between α-amylase and glucoamylase might contribute to the observed differences in hydrolysis among different starches.

### 3.2. Apparent amylose

The apparent amylose content (AAC) of native starches decreased after annealing (Table 2), which was attributed to amylose leaching out during the annealing process (Nakazawa & Wang, 2003). The AAC of all native and annealed non-waxy starches decreased during α-amylolysis. All native and annealed starches, except common corn, showed a continuous decrease in AAC for the first 15 h with slight or no decrease thereafter, while the AAC of common corn starch continued to decrease from 15 h to 36 h of hydrolysis. The initial more rapid decrease in AAC was assumed to result from hydrolysis of amylose in the amorphous lamellae, whereas the later decrease might be partly from the hydrolysis of amylose that was present in the crystalline lamellae. The decrease in AAC after 36 h was ~55% for common corn, ~22% for Hylon V, ~30 for Hylon VII, and ~30–40% for potato. The lower susceptibility of Hylon starches could be due to crystallization of hydrolyzed amylose during α-amylolysis, which impeded the further hydrolysis of amylose.

For glucoamylolysis, the AAC of native and annealed common corn did not change significantly for the first 15 h of hydrolysis, and thereafter gradually decreased. In contrast, a rapid increase in AAC was observed for native and annealed Hylon V, VII, and potato during the first 5 h of hydrolysis. The increase in AAC of Hylon V and VII could be due to their smaller molecular weight (MW) of amylose (Jane & Chen, 1992), which is more prone to crystallization during glucoamylolysis. The crystallization thereafter hindered the further hydrolysis by glucoamylase. In the meantime, amylopectin was preferentially hydrolyzed by glucoamylase, thus resulting in an increase in amylose ratio. On the other hand, the AAC was more than doubled in hydrolyzed potato starch, which could be ascribed to its substantially larger MW of amylose than that of Hylon and common corn amyloses (Jane & Chen, 1992). Thus more potato amylopectin might be hydrolyzed before amylose was degraded to become undetectable, consequently resulting in a higher AAC.

Table 2
Apparent amylose content (%, starch dry basis) of native and annealed starches after α-amylolysis and glucoamylolysis*

| | Duration (h) | α-Amylolysis | | Glucoamylolysis | |
|---|---|---|---|---|---|
| | | Native | Annealed | Native | Annealed |
| Common corn | 0 | 27.9[a] | 24.7[b] | 27.9[a] | 24.7[b] |
| | 5 | 22.1[b] | 17.0[c] | 23.9[a] | 25.0[a] |
| | 15 | 21.4[b] | 18.2[c] | 25.6[a] | 26.6[a] |
| | 36 | 12.8[b] | 10.3[c] | 19.1[a] | 18.5[a] |
| Hylon V | 0 | 52.7[a] | 48.5[b] | 52.7[a] | 48.5[b] |
| | 5 | 46.0[b] | 42.5[c] | 69.1[a] | 68.5[a] |
| | 15 | 42.7[a] | 40.4[a] | 67.6[a] | 66.0[a] |
| | 36 | 40.7[b] | 38.2[c] | 64.7[a] | 64.0[a] |
| Hylon VII | 0 | 70.5[a] | 67.3[b] | 70.5[a] | 67.3[b] |
| | 5 | 60.1[b] | 58.0[c] | 87.2[a] | 88.3[a] |
| | 15 | 50.3[b] | 48.5[c] | 82.2[a] | 84.3[a] |
| | 36 | 47.9[b] | 47.5[b] | 78.3[a] | 80.3[a] |
| Potato | 0 | 21.9[a] | 18.1[b] | 21.9[a] | 18.1[b] |
| | 5 | 20.1[c] | 15.7[d] | 46.3[a] | 42.1[b] |
| | 15 | 15.1[c] | 10.7[d] | 52.1[a] | 46.2[b] |
| | 36 | 15.2[c] | 10.8[d] | 48.5[a] | 44.7[b] |

* Means of two measurements followed by a common letter in the same row are not significantly different ($p < .05$).

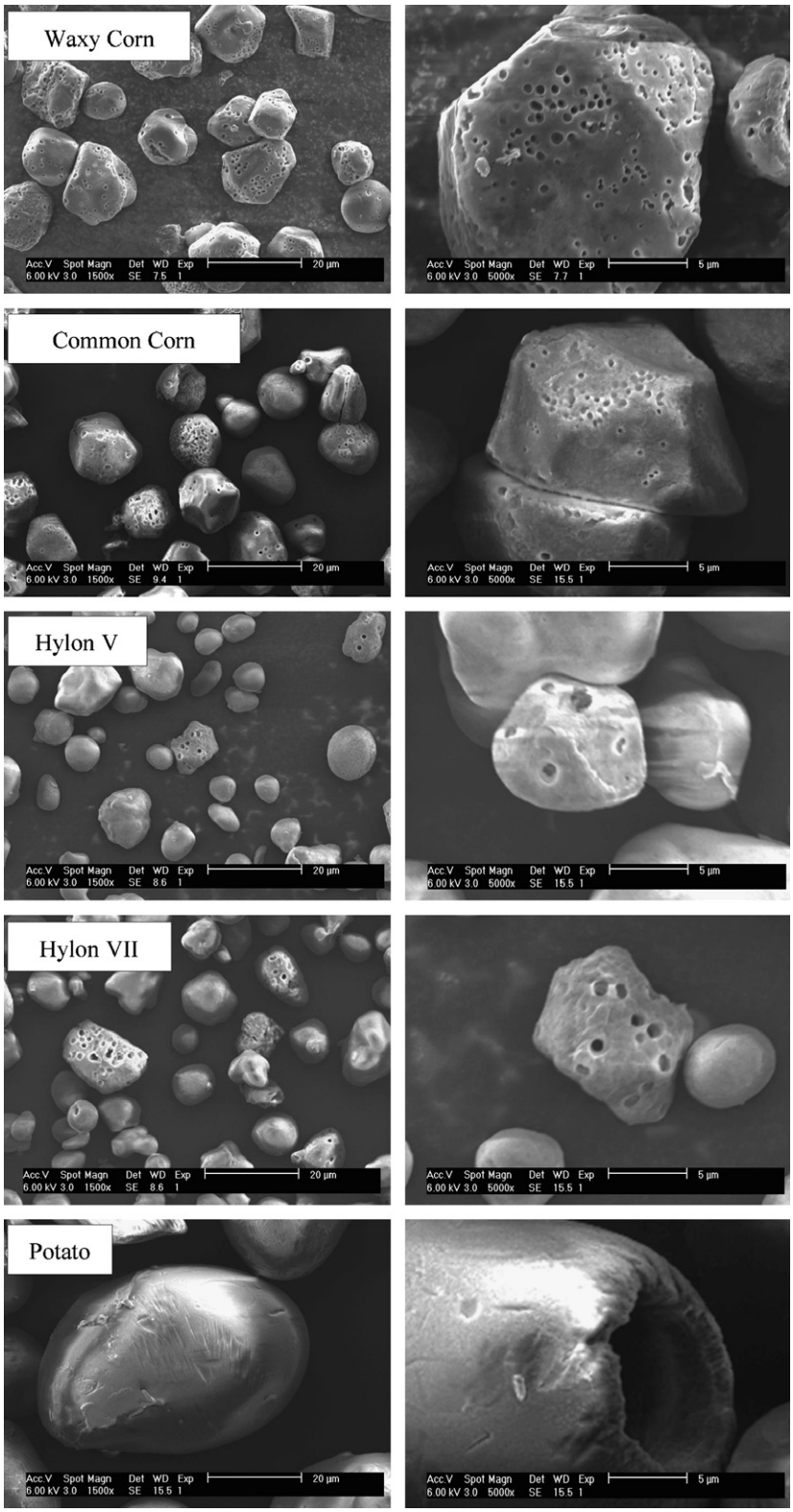


Fig. 2. SEM photographs of annealed waxy corn, common corn, Hylon V, Hylon VII, and potato starches hydrolyzed by α-amylase for 15 h.

### 3.3. Starch morphology

The representative SEM micrographs of hydrolyzed annealed starches by α-amylase and glucoamylase are presented in Figs. 2 and 3. The annealing treatment did not alter the appearance of hydrolyzed native starch granules (micrographs not shown). There was no difference with regard to patterns of enzymatic degradation between native

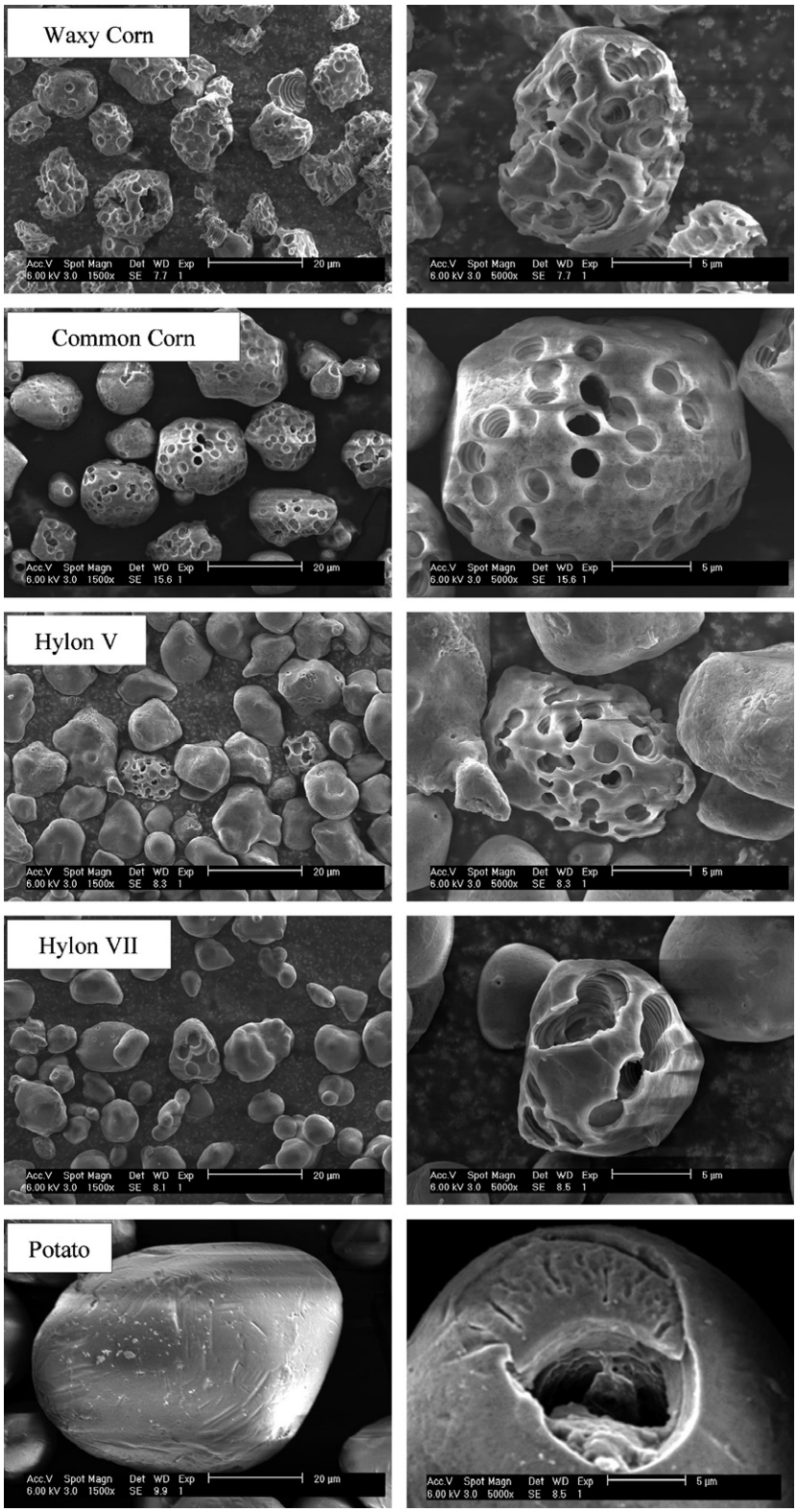


Fig. 3. SEM photographs of annealed waxy corn, common corn, Hylon V, Hylon VII, and potato starches hydrolyzed by glucoamylase for 15 h.

and annealed starches for both enzymes. For waxy and common corn starches, both α-amylase and glucoamylase appeared to hydrolyze starch granules via multiple attacks of localized digging, resulting in small pits into the granule. It appeared that the pits were initiated from the nonreducing ends of the molecules located on the surface of the granule. The presence and number of these hydrolyzed regions did not appear to be correlated with specific areas

Table 3
Gelatinization properties of hydrolyzed native and annealed starches by α-amylase and glucoamylase: $T_p$: peak gelatinization temperature; $T_c$–$T_o$: gelatinization temperature range (conclusion temperature – onset temperature); $\Delta H$: gelatinization enthalpy*

| Sample | | α-Amylolysis | | | | Glucoamylolysis | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 h | 5 h | 15 h | 36 h | 0 h | 5 h | 15 h | 36 h |
| *Waxy corn* | | | | | | | | | |
| Native | $T_p$ (°C) | 72.8$^{d}$ | 74.8$^{c}$ | 75.0$^{c}$ | 75.9$^{b}$ | 72.8$^{d}$ | 75.9$^{b}$ | 75.6$^{b}$ | 77.5$^{a}$ |
| | $T_c$–$T_o$ (°C) | 13.0$^{abc}$ | 13.8$^{ab}$ | 12.1$^{bcd}$ | 11.7$^{cde}$ | 13.0$^{abc}$ | 11.7$^{cde}$ | 10.3$^{ef}$ | 10.5$^{def}$ |
| | $\Delta H$ (J/g) | 14.7$^{a}$ | 14.6$^{a}$ | 15.0$^{a}$ | 14.0$^{a}$ | 14.7$^{a}$ | 12.3$^{b}$ | 11.1$^{c}$ | 11.8$^{b}$ |
| Annealed | $T_p$ (°C) | 77.0$^{c}$ | 78.6$^{b}$ | 79.1$^{b}$ | 79.2$^{a}$ | 77.0$^{c}$ | 76.7$^{b}$ | 80.9$^{a}$ | 81.4$^{a}$ |
| | $T_c$–$T_o$ (°C) | 6.6$^{cd}$ | 7.8$^{abc}$ | 7.7$^{abc}$ | 8.6$^{ab}$ | 6.6$^{cd}$ | 7.4$^{bcd}$ | 7.3$^{bcd}$ | 7.3$^{bcd}$ |
| | $\Delta H$ (J/g) | 17.3$^{a}$ | 15.2$^{b}$ | 14.7$^{b}$ | 12.8$^{c}$ | 17.3$^{a}$ | 13.1$^{c}$ | 10.5$^{d}$ | 10.4$^{d}$ |
| *Common corn* | | | | | | | | | |
| Native | $T_p$ (°C) | 72.3$^{f}$ | 73.6$^{e}$ | 74.9$^{bcd}$ | 74.8$^{bcd}$ | 72.3$^{f}$ | 74.6$^{cd}$ | 75.7$^{ab}$ | 75.5$^{abc}$ |
| | $T_c$–$T_o$ (°C) | 9.2$^{a}$ | 9.3$^{a}$ | 9.2$^{a}$ | 8.8$^{a}$ | 9.2$^{a}$ | 8.5$^{a}$ | 9.0$^{a}$ | 8.4$^{a}$ |
| | $\Delta H$ (J/g) | 12.3$^{ab}$ | 12.2$^{abc}$ | 12.5$^{ab}$ | 11.3$^{bcd}$ | 12.3$^{abc}$ | 10.5$^{d}$ | 8.9$^{e}$ | 9.1$^{e}$ |
| Annealed | $T_p$ (°C) | 77.4$^{b}$ | 78.5$^{a}$ | 78.8$^{a}$ | 78.3$^{a}$ | 77.4$^{b}$ | 78.2$^{a}$ | 78.4$^{a}$ | 78.8$^{a}$ |
| | $T_c$–$T_o$ (°C) | 6.5$^{bc}$ | 6.7$^{abc}$ | 6.6$^{bc}$ | 8.7$^{ab}$ | 6.5$^{bc}$ | 7.2$^{abc}$ | 8.2$^{abc}$ | 7.0$^{abc}$ |
| | $\Delta H$ (J/g) | 15.2$^{a}$ | 12.7$^{b}$ | 11.9$^{c}$ | 10.9$^{d}$ | 15.2$^{a}$ | 11.7$^{c}$ | 10.1$^{e}$ | 9.2$^{f}$ |
| *Hylon V* | | | | | | | | | |
| Native | $T_p$ (°C) | 76.8$^{a}$ | 78.1$^{a}$ | 78.1$^{a}$ | 79.2$^{a}$ | 76.8$^{a}$ | 79.9$^{a}$ | 79.2$^{a}$ | 78.9$^{a}$ |
| | $T_c$–$T_o$ (°C) | 37.6$^{ab}$ | 36.1$^{abc}$ | 36.2$^{abc}$ | 36.3$^{abc}$ | 37.6$^{ab}$ | 31.0$^{cd}$ | 34.3$^{bcd}$ | 35.2$^{abc}$ |
| | $\Delta H$ (J/g) | 16.8$^{a}$ | 21.0$^{a}$ | 20.3$^{a}$ | 19.4$^{a}$ | 16.8$^{a}$ | 20.6$^{a}$ | 20.3$^{a}$ | 20.5$^{a}$ |
| Annealed | $T_p$ (°C) | 77.2$^{b}$ | 83.4$^{a}$ | 83.4$^{a}$ | 83.8$^{a}$ | 77.2$^{b}$ | 83.4$^{a}$ | 82.3$^{a}$ | 84.0$^{a}$ |
| | $T_c$–$T_o$ (°C) | 31.8$^{a}$ | 30.2$^{a}$ | 30.1$^{a}$ | 29.3$^{a}$ | 31.8$^{a}$ | 27.9$^{a}$ | 30.0$^{a}$ | 30.4$^{a}$ |
| | $\Delta H$ (J/g) | 19.2$^{b}$ | 18.5$^{b}$ | 16.2$^{b}$ | 13.6$^{c}$ | 19.2$^{b}$ | 18.8$^{b}$ | 22.3$^{a}$ | 22.4$^{a}$ |
| *Hylon VII* | | | | | | | | | |
| Native | $T_p$ (°C) | 69.1$^{d}$ | 77.2$^{bc}$ | 79.5$^{abc}$ | 81.2$^{ab}$ | 69.1$^{d}$ | 81.7$^{ab}$ | 81.8$^{ab}$ | 82.0$^{ab}$ |
| | $T_c$–$T_o$ (°C) | 41.3$^{a}$ | 38.2$^{b}$ | 37.1$^{b}$ | 35.3$^{cd}$ | 41.3$^{a}$ | 33.3$^{de}$ | 34.9$^{cd}$ | 34.0$^{cde}$ |
| | $\Delta H$ (J/g) | 16.3$^{a}$ | 7.6$^{b}$ | 9.1$^{b}$ | 9.4$^{b}$ | 16.3$^{a}$ | 8.4$^{b}$ | 9.3$^{b}$ | 10.3$^{b}$ |
| Annealed | $T_p$ (°C) | 86.9$^{a}$ | 86.7$^{a}$ | 87.0$^{a}$ | 87.3$^{a}$ | 86.9$^{a}$ | 87.7$^{a}$ | 87.7$^{a}$ | 86.3$^{a}$ |
| | $T_c$–$T_o$ (°C) | 38.8$^{a}$ | 32.2$^{b}$ | 30.3$^{b}$ | 30.1$^{b}$ | 38.8$^{a}$ | 27.5$^{c}$ | 27.7$^{c}$ | 27.0$^{c}$ |
| | $\Delta H$ (J/g) | 20.1$^{a}$ | 13.0$^{b}$ | 12.3$^{b}$ | 13.6$^{b}$ | 20.1$^{a}$ | 17.0$^{a}$ | 17.2$^{a}$ | 18.3$^{a}$ |
| *Potato* | | | | | | | | | |
| Native | $T_p$ (°C) | 67.3$^{c}$ | 65.2$^{d}$ | 67.1$^{c}$ | 68.9$^{b}$ | 67.3$^{c}$ | 69.2$^{b}$ | 69.7$^{b}$ | 71.2$^{a}$ |
| | $T_c$–$T_o$ (°C) | 15.1$^{a}$ | 10.1$^{bc}$ | 9.1$^{bcd}$ | 10.0b$^{c}$ | 15.1$^{a}$ | 8.3$^{bcd}$ | 9.1$^{bcd}$ | 7.3$^{cd}$ |
| | $\Delta H$ (J/g) | 16.5$^{a}$ | 15.2$^{b}$ | 14.1$^{c}$ | 15.0$^{b}$ | 16.5$^{a}$ | 15.1$^{b}$ | 14.1$^{c}$ | 15.0$^{b}$ |
| Annealed | $T_p$ (°C) | 77.4$^{a}$ | 71.2$^{e}$ | 72.5$^{bcd}$ | 72.5$^{bcd}$ | 77.4$^{a}$ | 72.6$^{bcd}$ | 72.0$^{cd}$ | 72.8$^{bc}$ |
| | $T_c$–$T_o$ (°C) | 7.3$^{bc}$ | 8.3a$^{bc}$ | 9.2$^{abc}$ | 9.8$^{ab}$ | 7.3$^{bc}$ | 7.8$^{bc}$ | 8.6$^{abc}$ | 7.8$^{bc}$ |
| | $\Delta H$ (J/g) | 19.2$^{a}$ | 16.0$^{bc}$ | 15.1$^{bcd}$ | 15.3$^{bc}$ | 19.2$^{a}$ | 14.9$^{bcd}$ | 14.0$^{cd}$ | 15.6$^{bc}$ |

* Means of two measurements followed by a common letter in the same row are not significantly different ($p < .05$).

on granules or with specific types of granules. Similar degradation patterns were observed in starches during glucoamylolysis except that pits were larger and deeper into granules as a result of more extensive hydrolysis (Table 1). For Hylon starches only a few granules were noted with pits from limited hydrolysis (Table 1).

The mode of enzymatic attack of potato starch differed from the extensive digging observed in corn starches. Hydrolyzed potato starch showed a single hole on one end of the granule with more extensive hydrolysis of the internal regions of the granule, which agrees with the findings by Wang et al. (1997). They observed that the internal structure of annealed sago starch was rapidly digested by α-amylase and glucoamylase, followed by slow surface erosion. Recently Lin et al. (2006) reported that the end distant from the hilum of native lotus starch was more susceptible to α-amylolysis. Digestion by enzymes would affect the loosely packed internal region of the granule faster than the densely packed periphery, thus leaving an empty shell. They concluded that this degradation pattern was due to heterogeneous molecular organization.

### 3.4. Thermal properties

The gelatinization properties of native and annealed starches and their granular residues after 5, 15, and 36 h of hydrolysis by both enzymes as measured by DSC are listed in Table 3. Native and annealed waxy and common

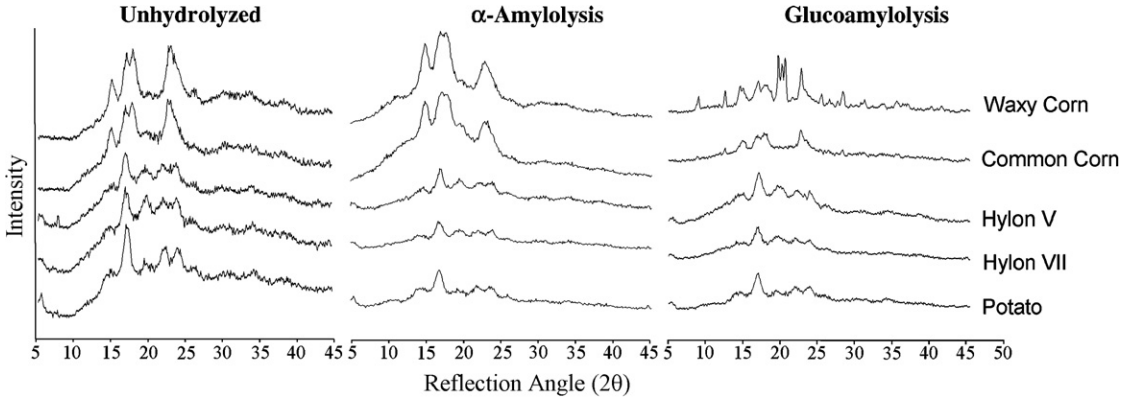


Fig. 4. X-ray diffraction patterns of unhydrolyzed and hydrolyzed annealed waxy corn, common corn, Hylon V, Hylon VII, and potato starches by α-amylase for 36 h.

Table 4
Relative crystallinity (%) of native and annealed starches after α-amylolysis and glucoamylolysis for 36 h*

| Starch | Unhydrolyzed | | α-Amylase | | Glucoamylase | |
|---|---|---|---|---|---|---|
| | Native | Annealed | Native | Annealed | Native | Annealed |
| Waxy corn | 28.7$^{b}$ | 29.9$^{b}$ | 29.6$^{b}$ | 30.7$^{b}$ | 44.8$^{a}$ | 46.5$^{a}$ |
| Common corn | 23.9$^{c}$ | 24.5$^{c}$ | 20.0$^{d}$ | 20.5$^{d}$ | 29.9$^{b}$ | 38.9$^{a}$ |
| Hylon V | 28.7$^{a}$ | 28.6$^{a}$ | 29.1$^{a}$ | 24.9$^{b}$ | 22.6$^{c}$ | 20.8$^{c}$ |
| Hylon VII | 29.2$^{ab}$ | 28.2$^{ab}$ | 24.3$^{cd}$ | 28.8$^{abc}$ | 25.1$^{cd}$ | 26.2$^{bcd}$ |
| Potato | 36.6$^{b}$ | 38.1$^{a}$ | 33.6$^{c}$ | 33.1$^{c}$ | 32.9$^{c}$ | 29.4$^{d}$ |

* Means of two measurements followed by a common letter in the same row are not significantly different ($p < .05$).

corn and native Hylon VII and potato exhibited increased peak gelatinization temperatures ($T_p$) and decreased gelatinization enthalpy ($\Delta H$) on hydrolysis. There was no significant change in $T_p$ and $\Delta H$ for native Hylon V during hydrolysis by both enzymes. Annealed potato starch was the only starch that showed a decrease in $T_p$ on hydrolysis. Most starches displayed either decreased or unchanged gelatinization temperature ranges (conclusion – onset temperature) during the course of hydrolysis with the exception of annealed waxy corn.

The increase in $T_p$ indicates hydrolysis of the amorphous structure by both enzymes because the amorphous regions facilitate the melting of crystalline structure. The decrease in $\Delta H$ on the other hand supports the hydrolysis of the crystalline and helical structures. Therefore, the present results suggest simultaneous hydrolysis of both amorphous and crystalline structures of native and annealed starches by both enzymes. The $T_p$ of potato starch showed the most increase after annealing from 67.3 °C to 77.4 °C among the starches, suggesting a highly improved crystalline structure after annealing. The formation of enhanced ordered structures allowed for a significant increase in the more porous structures, which might subsequently promote more rapid hydrolysis the crystalline structures by enzymes, thus resulting in reduced $T_p$ on hydrolysis. Starches hydrolyzed by glucoamylase generally exhibited higher $T_p$, narrower gelatinization temperature ranges, and lower $\Delta H$ values than those hydrolyzed by α-amylase, assuming that the higher degree of hydrolysis by glucoamylase manifested changes in gelatinization properties.

### 3.5. X-ray diffraction

The X-ray diffraction patterns of annealed starches before and after 36 h of hydrolysis by α-amylase and glucoamylase are presented in Fig. 4. The X-ray diffraction patterns of native starches were similar to their annealed counterparts; therefore their results are not shown. The native and annealed starches displayed typical A-type pattern for waxy corn and common corn with main peaks at 15°, 17°, 18°, and 23°, and B-type pattern for Hylon V, VII, and potato with main peaks at 5.6°, 14.4°, 17°, and 22°, and 24° (Zobel, 1964). Upon hydrolysis, all main peaks decreased in intensity but the extent of decrease varied. For waxy and common corn, the intensity of the main peaks decreased slightly during α-amylolysis, but noticeably during glucoamylolysis. In contrast, the intensity of the main peaks in Hylon V, VII, and potato significantly reduced on hydrolysis, but the profiles and peak intensities were similar regardless of enzymes. The peak at 20° is characteristic for formation of amylose–lipid complex and became more visible on hydrolysis for common corn and Hylon starches. Waxy starch showed a major triplet peak at 20° after glucoamylolysis. The X-ray diffraction patterns clearly showed the reduction in peak intensity as well as in amorphous area. Therefore, these results provide direct evidences of simultaneous degradation of the amorphous as

well as the crystalline structures by α-amylase and glucoamylase.

The relative crystallinity of native and annealed starches either unchanged or decreased during α-amylolysis, but those of waxy and common corn increased and those of Hylon V, VII, and potato decreased during glucoamylolysis (Table 4). There was no difference in relative crystallinity for starches after annealing by both enzymes, except Hylon V by α-amylase and potato by glucoamylase. More crystalline structure was hydrolyzed in annealed Hylon V by α-amylase and in annealed potato by glucoamylase.

## 4. Conclusions

Annealed starches exhibited different properties from native ones during α-amylolysis: higher degree of hydrolysis (potato and Hylon V and VII), lower AAC (potato), higher $T_p$ (all starches), and lower relative crystallinity (Hylon V). During α-glucoamylolysis all annealed starches displayed higher $T_p$, and annealed potato showed an increase in degree of hydrolysis and relative crystallinity when compared with the native ones. The results of gelatinization and X-ray diffraction supported the simultaneous degradation of both amorphous and crystalline structures during α-amylolysis and glucoamylolysis. Annealing promoted the formation of more porous structures to allow for enhanced enzyme hydrolysis, which significantly change some physicochemical properties such as gelatinization temperature but the extent of change was affected by type of starch and enzyme.

## References


Baldwin, P. M., Adler, J., Davies, M. C., & Melia, C. D. (1998). High Resolution imaging of starch granule surfaces by atomic force microscopy. *Journal of Cereal Science, 27*(3), 255–265.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Biochemistry, 13*, 367–374.

Franco, C. M. L., Ciacco, C. F., & Tavares, D. Q. (1988). Studies on the susceptibility of granular cassava and corn starches to enzymatic attack. Part 2: Study of the granular structure of starch. *Starch – Stärke, 40*(1), 29–32.

Gallant, D., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: Evidence of new level of granular organization. *Carbohydrate Polymers, 32*, 177–191.

Gallant, D., Bouchet, B., Buleon, A., & Perez, S. (1992). Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European Journal of Clinical Nutrition, 46*, S3–S16.

Hoover, R., & Vasanthan, T. (1994). The effect of annealing on the physiochemical properties of wheat, oat, potato and lentil starches. *Journal of Food Biochemistry, 17*, 303–325.

Jacobs, H., Eerlingen, R. C., Rouseu, N., Colonna, P., & Delcour, J. A. (1998). Acid hydrolysis of native and annealed wheat, potato and pea starches-DSC melting features and chain length distributions of lintnerised starches. *Carbohydrate Research, 308*, 359–371.

Jacobs, H., Mischenko, N., Koch, M. H. J., Eerlingen, R. C., Delcour, J. A., et al. (1998). Evaluation of the impact of annealing on gelatinization at intermediate water content of wheat and potato starches: A differential scanning calorimetry and small angle X-ray scattering study. *Carbohydrate Research, 306*(1–2), 1–10.

Jane, J.-L., & Chen, J.-F. (1992). Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chemistry, 69*(1), 60–65.

Jane, J.-L., Wong, K.-S., & McPherson, A. E. (1997). Branch-structure difference in starches of A- and B-type X-ray patterns revealed by their Naegeli dextrins. *Carbohydrate Research, 300*, 219–227.

Juliano, B., Perez, C., Blakeney, A., Castillo, D., Kongseree, N., Laignelet, B., et al. (1981). International cooperative testing on the amylose content of milled rice. *Starch/Stärke, 33*(5), 157–162.

Kimura, A., & Robyt, J. F. (1995). Reaction of enzymes with starch granules: Kinetics and products of the reaction with glucoamylase. *Carbohydrate Research, 222*, 87–107.

Kimura, A., & Robyt, J. F. (1996). Reaction of enzymes with starch granules: Enhanced reaction of glucoamylase with gelaitnized starch granules. *Carbohydrate Research, 288*, 233–240.

Kiseleva, V. I., Krivandin, A. V., Fornal, J., Blaszczak, W., Jelinski, T., & Yuryev, V. P. (2005). Annealing of normal and mutant wheat starches. LM, SEM, DSC, and SAXS studies. *Carbohydrate Research, 340*, 75–83.

Knutson, C. A. (1990). Annealing of maize starches at elevated temperatures. *Cereal Chemistry, 67*(4), 376–385.

Kohyama, K., & Sasaki, T. (2006). Differential scanning calorimetry and a model calculation of starches annealed at 20 and 50C. *Carbohydrate Polymers, 63*, 82–88.

Lauro, M., Forssell, P. M. P., Suortti, M. T., Hulleman, S. H. D., & Poutanen, K. S. (1999). a-Amylolysis of large barley starch granules. *Cereal Chemistry, 76*(6), 925–930.

Lauro, M., Suortti, T., Autio, K., Linko, P., & Poutanen, K. (1993). Accessibility of barley starch granules to alpha-amylase during different phases of gelatinization. *Journal of Cereal Science, 17*, 125–136.

Leach, H., & Schoch, T. (1961). Action of various amylases on granular starches. *Cereal Chemistry, 38*, 34–46.

Lin, H.-M., Chang, Y.-H., Lin, J.-H., Jane, J.-L., Sheu, M.-J., & Lu, T.-J. (2006). Heterogeneity of lotus rhizome starch granules as revealed by a-amylase degradation. *Carbohydrate Polymers, 66*, 528–536.

Nakazawa, Y., & Wang, Y.-J. (2003). Acid hydrolysis of native and annealed starches and branch-structure of their Naegeli dextrins. *Carbohydrate Research, 338*(24), 2871–2882.

Nakazawa, Y., & Wang, Y.-J. (2004). Effect of annealing on starch-palmitic acid interaction. *Carbohydrate Polymers, 57*(3), 327–335.

Robyt, J. F., & French, D. (1963). Action pattern and specificity of an amylase from *Bacillus subtilis*. *Archives of Biochemistry and Biophysics, 100*, 451–467.

Stoffer, B., Frandsen, T. P., Busk, P. K., & Schneider, P. (1993). Production, purification and characterization of the catalytic domain of glucoamylase from *Aspergillus niger*. *Biochemistry Journal, 292*, 197–202.

Stute, R. (1992). Hydrothermal modification of starches: The difference between annealing and heat/moisture-treatment. *Starch/Stärke, 44*(6), 205–214.

Svensson, B., Larsen, K., Svendsen, I., & Boel, E. (1983). The complete amino acid sequence of the glycoprotein, glucoamylase G1, from *Aspergillus niger*. *Carlsberg Research Communications, 48*(6), 529–544.

Tester, R. F., & Morrison, W. R. (1990). Swelling and gelatinization of cereal starch. I. Effects of amylopectin, amylose, and lipids. *Cereal Chemistry, 67*, 551–557.

Waduge, R. N., Hoover, R., Vasanthan, T., Gao, J., & Li, J. (2006). Effect of annealing on the structure and physicochemical properties of barley starches of varying amylose content. *Food Research International, 39*(1), 59–77.

Wang, W. J., Powell, A. D., & Oates, C. G. (1997). Effect of annealing on the hydrolysis of sago starch granules. *Carbohydrate Polymers, 33*, 195–202.

Yook, C., & Robyt, J. F. (2002). Reactions of alpha amylases with starch granules in aqueous suspension giving products in solution and in a minimum amount of water giving products inside the granule. *Carbohydrate Research, 337*, 1113–1117.

Yost, D. A., & Hoseney, R. C. (1986). Annealing and glass transition of starch. *Starch/Stärke, 38*(9), 289–292.

Zhou, Y., Hoover, R., & Liu, Q. (2004). Relationship between alpha-amylase degradation and the structure and physiochemical properties of legume starches. *Carbohydrate Polymers, 57*, 299–317.

Zobel, H. F. (1964). X-ray analysis of starch granules. In R. L. Whistler, R. J. Smith, & J. N. BeMiller (Eds.). *Methods in carbohydrate chemistry* (Vol. 4, pp. 109–113). Orlando, FL: Academic Press, Inc.