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Starch from hull-less barley: V. In-vitro susceptibility of waxy, normal, and high-amylose starches towards hydrolysis by alpha-amylases and amyloglucosidase

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Abstract

Waxy (CDC Alamo), normal (CDC Dawn), and high-amylose (SB 94893) hull-less barley (HB) starches were isolated and their susceptibilities to porcine pancreatic α-amylase (PPA), *Bacillus species* α-amylase (BAA), and *Aspergillus niger* amyloglucosidase (AAG) were determined. Scanning and transmission electron micrographs showed that the patterns of enzyme hydrolysis (surface erosion, endoerosion, and the erosion at the equatorial groove plane) were dependent on the enzyme source and starch types. The outer layers of normal and high-amylose HB starch granules were more resistant to enzyme hydrolysis. The hydrolysis rate of waxy HB starch by the three amylases at 37 °C was significantly higher than those of normal and high-amylose HB starches. The degree of hydrolysis by PPA, after 72 h, reached 91–97% in all three starches. However, BAA and AAG showed significantly lower degrees of hydrolysis in normal (<30%) and high-amylose (<37%) HB starches than in waxy HB starch (>67%). Glucose, maltose, and maltotriose contents of the hydrolysates, collected at different time intervals, were determined by HPLC. The concentration and composition of soluble sugars in hydrolysates varied with enzyme source, starch types, and condition of hydrolysis. The study suggested that both morphological and ultrastructural features influence in-vitro hydrolysis and the ultrastructure of waxy HB starch may be more open than those of normal and high-amylose HB starches.

Keywords: Hull-less barley; Starch; Enzyme hydrolysis; SEM; TEM

1. Introduction

In-vitro enzyme hydrolysis of starches is not only an important industrial process for production of sweeteners, syrups, and chemicals (e. g. ethanol, acetone, and lactic acid) (Nigam & Singh, 1995), but also as a probe for studying starch ultrastructure (French, 1984; Gallant, Bouchet, & Baldwin, 1997). Numerous researchers have investigated enzyme hydrolysis of starches from cereals, roots, tubers, and legumes in terms of enzyme adsorption, action pattern, extent of hydrolysis, degree of crystallinity, and hydrolysis products. The susceptibility of starch to hydrolysis by amylases has been shown to vary with enzyme source and starch origin (Colonna, Leloup, & Buléon, 1992; Gallant, Bouchet, Buléon, & Pérez, 1992; Gérard, Colonna, Buléon, & Planchot, 2001; Hoover, 2001; Hoover & Sosulski, 1991; Koba, Saha, & Ueda, 1986; Lauro, Suortti, Autio, Linko, & Poutanen, 1993; Leach & Schoch, 1961; Planchot, Colonna, Gallant, & Bouchet, 1995; Rasper, Perry, & Duitschaever, 1974; Rickard, Asaoka, & Blanshard, 1991).

Electron microscopy (Evers & McDermott, 1970; Fuwa, Sugimoto, & Takaya, 1979; Fuwa, Sugimoto, Tanaka, & Glover, 1978; Gallant, Mercier, & Guilbot, 1972; Helbert, Schülein, & Henrissat, 1996; MacGregor & Ballance, 1980; Valetudie, Colonna, Bouchet, & Gallant, 1993; Planchot et al., 1995) has revealed that α-amylase pits the granule surface first and then penetrates through

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pinholes/internal channels and then hydrolyzes the granule from inside-out. The A-, B- and C-types of starches (as classified according to X-ray diffraction pattern), show different susceptibilities to α-amylase hydrolysis. Generally, A-type (most cereals and tapioca) starches are more readily hydrolyzed by α-amylase than B-type (amylomaize and potato) starches (Gallant et al., 1972; Planchot et al., 1995). Waxy starches are hydrolyzed by α-amylase faster than non-waxy starches (Leach & Schoch, 1961; MacGregor & Ballance, 1980). For a given starch, the rate of hydrolysis is dependent on enzyme type (Planchot et al., 1995; Valetudie et al., 1993), condition of hydrolysis (e.g. concentration, pH, and temperature) (Franco & Ciacco, 1987), and physical (Kurakake, Tachibana, Masaki, & Komaki, 1996; Lauro et al., 1993) and chemical (Tharanathan & Ramadas Bhat, 1988; Wolf, Bauer, & Fahey Jr., 1999) modification of starch prior to hydrolysis. Kimura and Robyt (1995) have shown that variations in susceptibility of cereal and tuber starches towards amyloglucosidase can be partly explained by differences in their ultrastructure. The ultrastructural features of granule organization include inter-chain association (e.g. amylose-amylose and/or amylose-amylopectin) (Vasanthan & Bhatty, 1996), type and degree of crystallinity (double helical packing) (Gérard et al., 2001), the number of the crystalline and amorphous lamellae in each growth ring (Gallant, Bouchet, & Baldwin, 1997; Li, Vasanthan, Hoover, & Rossnagel, 2003), and amylose-lipid interaction (Anger, Richter, Kettlitz, & Radosta, 1994; Appelqvist & Debet, 1997; Lauro, Forssell, Suortti, Hulleman, & Poutanen, 1999; Morrison, 1995). Colonna, Buléon, and Lemarié (1988) have shown, by iodine binding study, that there is no preferential hydrolysis of either amylose or amylopectin by α-amylase. X-ray diffraction has also shown that both amorphous and semi-crystalline regions of barley starch granules are hydrolyzed simultaneously by α-amylase at the initial stage of hydrolysis and lipid-complexed amyloses were retained in the residue of starches even after about half of the starch was solublized (Lauro et al., 1999). Gérard et al. (2001) further reported that amorphous material co-exists with B-type crystallites in hydrolysis (α-amylase) residues of maize mutant starches (with a wide range of crystallinity levels and various ratios of A-, B-, and V- crystalline types) although the ultimate extent of hydrolysis were highly correlated with the amount of B-type crystallites. These studies suggest that there is a strong molecular association in the residue (mainly in the semi-crystalline regions) of the starch granule.

Starch from hull-less barley (HB) has comparable properties with maize starch (Li, Vasanthan, Rossnagel, & Hoover, 2001a, 2001b; Vasanthan & Bhatty, 1996). There is a great potential for HB starch to replace industrial applications of maize starch, which is widely used currently in North America. However, studies on enzyme hydrolysis of HB starches by various amylases and visualization of structural changes during hydrolysis have not been well documented. The objective of this study was two fold: (1) a comparison of the susceptibilities of starches from HB genotypes (waxy, normal and high-amylose) toward α-amylases and amyloglucosidase and, (2) to obtain an understanding of how HB starch ultrastructure influence the rate and extent of amylase hydrolysis.

2. Materials and methods

2.1. Starch sources

Waxy (zero-amylose, CDC Alamo), normal (CDC Dawn), and high-amylose (SB 94893) HB grains (grown at the same location and harvested at Saskatoon in 1998) were obtained from the Crop Development Center, University of Saskatchewan, Saskatoon, Canada. Barley grains were dry ground in a UDY cyclone sample mill equipped with a 0.5 mm screen. Starch was isolated from ground barley grains as described previously (Li et al., 2001a).

2.2. Enzymes

α-Amylase (suspension in 2.9M NaCl solution containing 3 mM $CaCl_2$, 37 mg protein/ml) from porcine pancreas (EC 3.2.1.1) (PPA), α-amylase (crystallized, lyophilized powder, 2150 units/mg solid) from *Bacillus species* (EC 3.2.1.1) (BAA), and amyloglucosidase (lyophilized powder containing less than 0.02% glucose, 40 units/mg solid) from *Aspergillus niger* (EC 3.2.1.3) (AAG) with specific activities of 1020, 2880, and 42 units/mg protein, respectively, were purchased from Sigma Chemical Co. (St. Louis, MO).

2.3. Enzyme hydrolysis

Duplicate samples of starch (120±0.1 mg) were suspended in 10 ml of 0.1 M phosphate buffer (pH 6.9) containing 0.006 M NaCl. The screw cap tubes containing the starch samples were placed in a constant temperature shaking water bath at 37 °C. Then, PPA (16.5 μl, 5 units/mg starch) and BAA (45 μl, 5 units/mg starch) for PPA and BAA hydrolysis, respectively, were added to the starch slurry. Hydrolysis was conducted for 72 h. For AAG hydrolysis, starch (120±0.1 mg) was suspended in 0.05 M acetate buffer (pH 4.5) followed by the addition of 0.1 ml of buffered AAG (2.5 units/mg starch). Hydrolysis was conducted for 72 h at 55 °C. For all three enzymes, 1 ml of aliquots were removed at specified time intervals and centrifuged (1700×*g*) for 5 min. Aliquots of the supernatant were analyzed for soluble carbohydrates (Bruner, 1964). Percentage of

hydrolysis was calculated as the amount (mg) of maltose released per 100 mg of dry starch. The residue was washed with distilled water (×3) and 100% ethanol (×2), then dried at 40 °C. The dried samples were used for electron microscopy.

2.4. Quantification of glucose, maltose, and maltotriose

The soluble products of hydrolysis (glucose, maltose, and maltotriose) were analyzed by high performance liquid chromatography (HPLC) with a Supelcosil LC-NH2-5um column (Supelcosil, Bellefonte, PA, USA), following the method of Vasanthan, Yeung, and Hoover (2001). Standard solutions of fructose, glucose, sucrose, maltose, and maltotriose were used for calibration.

2.5. Scanning electron microscopy

The dried starch sample from enzyme hydrolysis was directly mounted on circular aluminum stubs with double-sided sticky tape, coated with 12 nm gold, then examined and photographed in a JEOL (JSM 6301FXV) scanning electron microscope (JEOL, LTD, Tokyo, Japan) at an accelerating voltage of 5 kV.

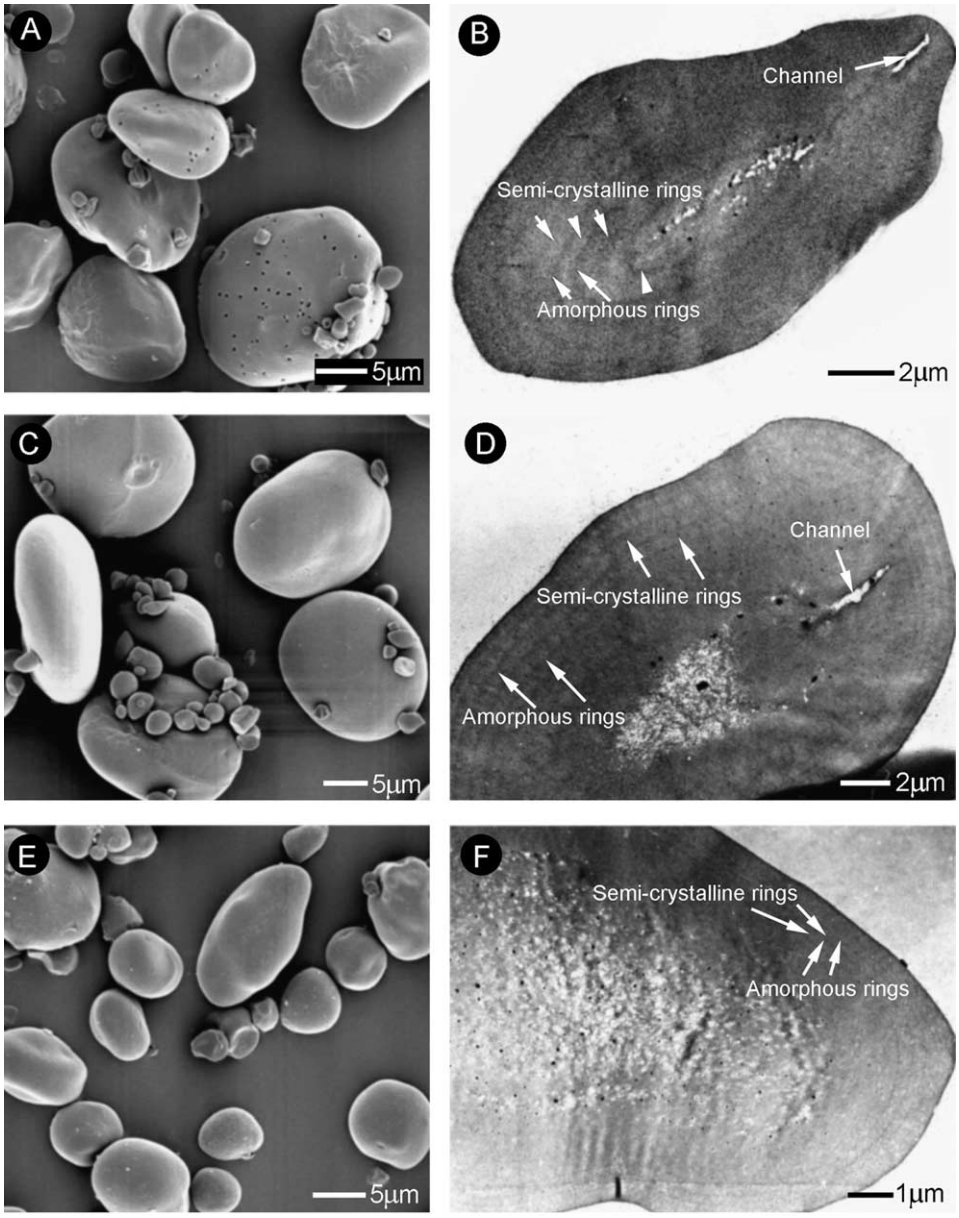

Fig. 1. Scanning (A, C & E) and transmission (B, D & F) electron micrographs of HB starch granules of waxy (CDC Alamo) (A & B), normal (CDC Dawn) (C & D), and high-amylose (SB 94893) (E & F) types.

2.6. Transmission electron microscopy

The periodic acid-thiosemicarbazide-silver reaction (PATAg) method (Garcia, Colonna, Bouchet, & Gallant, 1997) was used to reveal ultra-structure of starch granules. Heavy metal ions (silver) can bind to oxidized (by periodic acid) starch molecules after specific oxidation of α-glycols at C2–C3 of the anhydro-glucose units of starch and thiosemicarbazide fixation, which gives good contrast on ultra-thin sections of granules under transmission electron microscope. Since oxidation is more efficient in the starch amorphous regions, the silver ions preferentially bind to the amorphous regions of oxidized starch granules, resulting in dark regions in transmission electron microscopy images, whilst semi-crystalline regions appear lighter.

Native starches and starch samples from enzyme hydrolysis were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 h at room temperature and then for 6 h at 4 °C. Fixed samples were pre-embedded in agar aqueous solution (3%), cut into small cubes (1 mm^3), and re-fixed for 1 h. After fixation, the sample cubes were washed in buffer (×2) and in distilled water (×2) for 20 min. For blocking residue free aldehyde, the washed sample cubes were immersed in a saturated 2,4-dinitrophenylhydrazine in 15% acetic acid solution for 1 h at room temperature. After washing (×4) in distilled water for 15 min, the sample cubes were oxidized in a 1% aqueous periodic acid solution for 45 min, washed again as above, and then immersed in saturated aqueous thiosemicarbazide solution for 24 h. The sample cubes were washed again and stained in 1% aqueous silver nitrate solution for 3 days in darkness, with daily changes of the staining solution. The stained cubes were rinsed with distilled water, dehydrated in an ethanol series (30–100%), and then embedded in EMbed 812 resin (EMS Inc., Fort Washington, PA). Controls of oxidative reaction were performed in parallel by substituting the periodic acid with 10% H_2O_2 (bleaching reagent). The embedded sample blocks were randomly cut into 90–100 nm thin sections with a diamond knife in an RMC (Tucson, AZ, USA) MT 4000 ultramicrotome. The ultra-thin sections collected on collodion-coated copper grids were observed and photographed with a Philips CM12 transmission electron microscope (F.E.I. Company, Tacoma, WA) at 60 kV without further staining.

3. Results and discussion

3.1. Granule morphology of native HB starches

Scanning and transmission electron micrographs of granules from native HB starches are presented in Fig. 1. The granule size of the three HB starches (Fig. 1A, C &

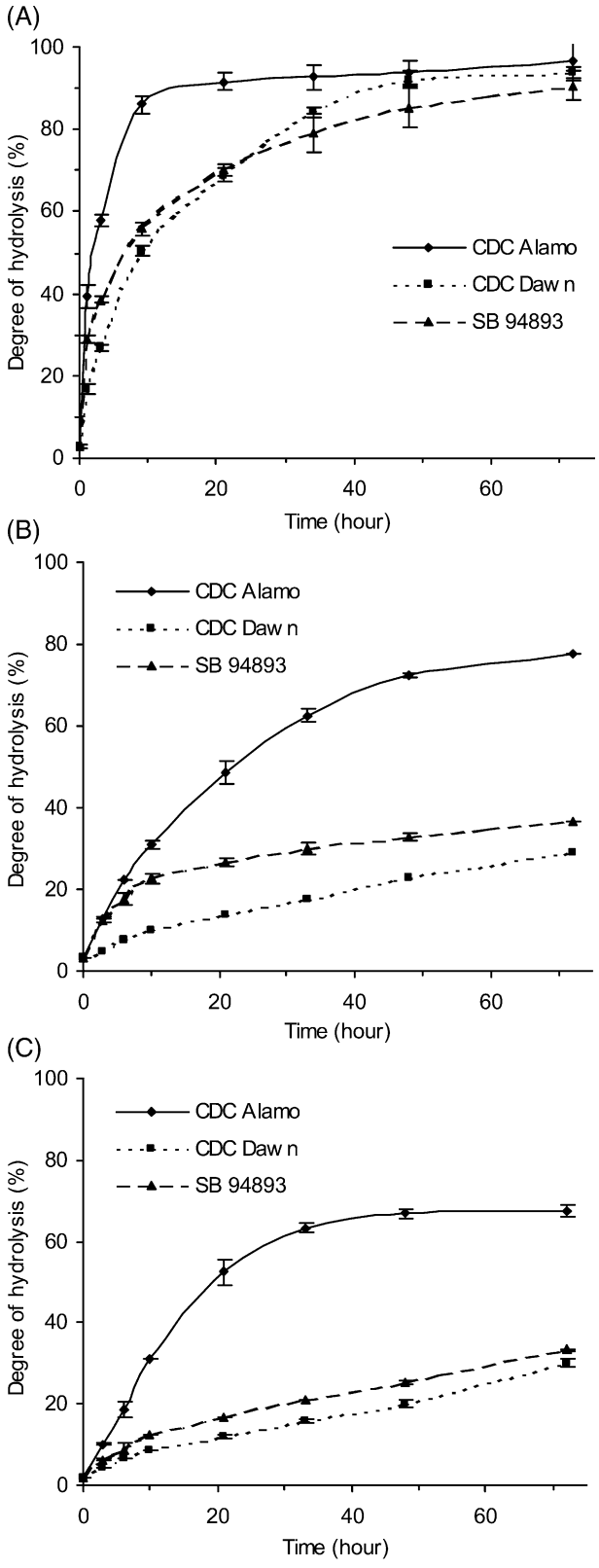

Fig. 2. Hydrolysis of HB starches of waxy (CDC Alamo), normal (CDC Dawn) and high-amylose (SB 94893) types by PPA (A), BAA (B) and AAG (C).

E) ranged from 2 to 30 μm in diameter. Granules were round to oval in shape with smooth surfaces. High-amylose HB starch showed smaller granules than waxy or normal HB starches. Numerous pinholes (up to 0.9 μm in diameter) were observed on larger waxy HB starch granules (Fig. 1A). A number of tiny pinholes were visible on the surface of normal HB starch granules (Fig. 1C); however, pinholes were rarely visible on the surface of high-amylose HB starch granules (Fig. 1E). Under the transmission electron microscope, the alternating growth rings (amorphous regions appeared as dark rings and semi-crystalline regions appeared as light rings) of waxy, normal and high-amylose types of HB starches were seen to vary in number and size (Fig. 1 B, D & F). Some internal channels were also occasionally observed in the thin-sections of waxy (Fig. 1 B) and normal (Fig. 1 D) HB starch granules.

3.2. Hydrolysis kinetics

The hydrolysis by α-amylase from porcine pancreas (PPA), α-amylase from *Bacillus species* (BAA), and amyloglucosidase from *Aspergillus niger* (AAG), in all three starches, was bi-phasic, a relatively rapid rate at the initial stage, followed by a progressively decreased rate thereafter (Fig. 2). The rate of hydrolysis at the initial stage varied with enzyme and starch types. During the time course of hydrolysis, waxy HB starch was more

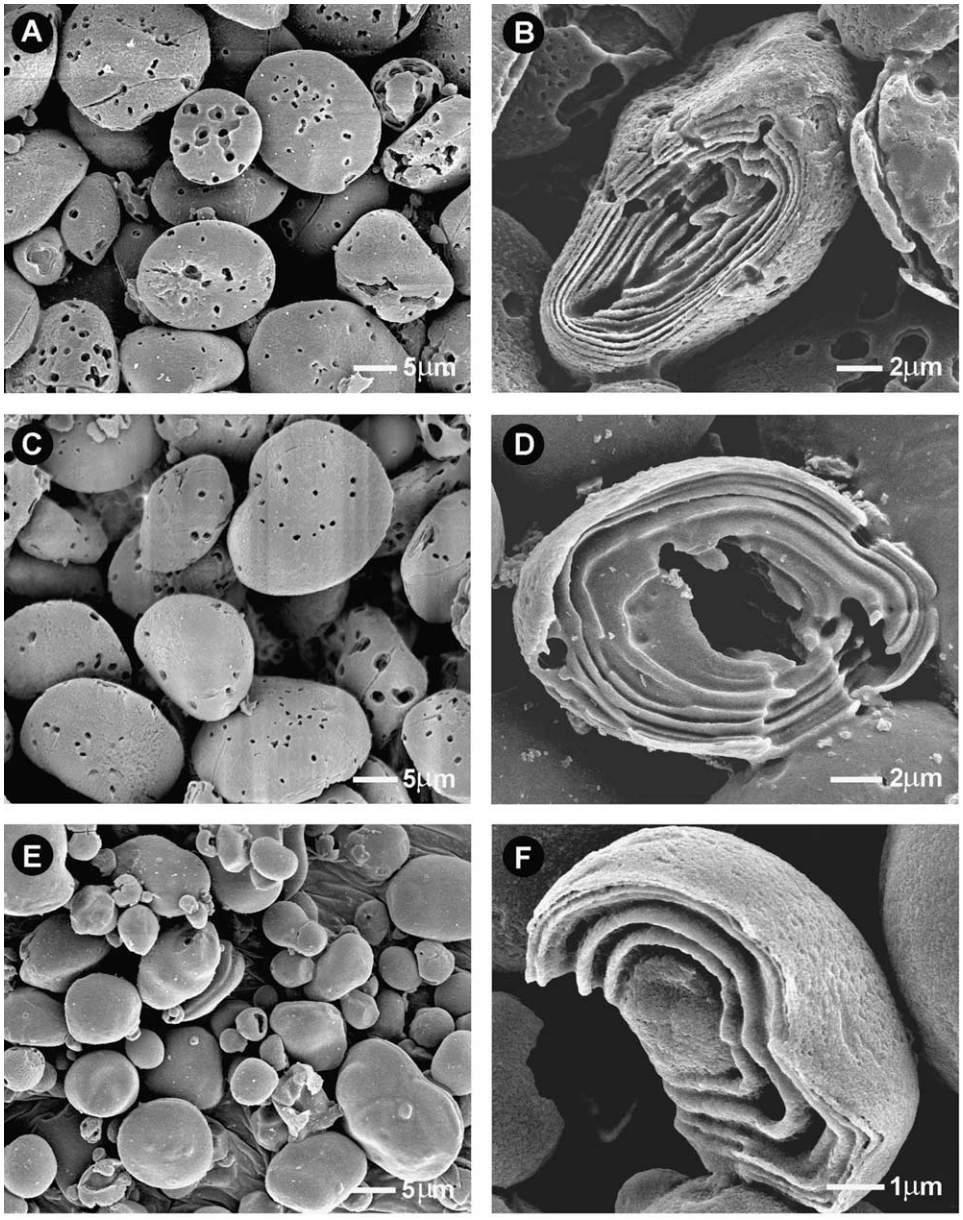

Fig. 3. Scanning electron micrographs of HB starch granules of waxy (CDC Alamo) (A & B), normal (CDC Dawn) (C & D), and high-amylose (SB 94893) (E & F) types hydrolyzed by PPA at 37 °C for 1 h.

readily hydrolyzed (by all three enzymes) than those from normal and high-amylose HB starches (Fig. 2A, B & C), while normal HB starch showed the lowest hydrolysis rate toward all three enzymes. After 72 h, waxy, normal and high-amylose HB starches were hydrolyzed to the extent of 97, 94, and 91%, respectively, by PPA, whereas, during the same time interval, the degrees of hydrolysis by BAA and AAG reached 78 and 67% in waxy HB starch, 29 and 30% in normal HB starch, and 37 and 33% in high-amylose HB starch, respectively.

Amylase hydrolysis involves an enzyme in solution acting on a solid starch substrate. Thus, the surface area accessible to enzyme and the efficiency of adsorption of enzyme onto this surface are critical kinetic parameters (Bertoft & Manelius, 1992). However, a wide variation occurs between enzyme adsorption and hydrolysis rate among starches of various origins and different types (Bertoft & Manelius, 1992; Kimura & Robyt, 1995). The rate of hydrolysis may be influenced by both the surface features and internal structure of starch granules. Knutson, Khoo, Cluskey, and Inglett (1982) studied the PPA hydrolysis of maize starch granule fractions according to granule size. They found that the hydrolysis rate for waxy, normal, and high-amylose maize starches was proportional to the surface area of granules, which may be closely related to the adsorption of enzyme onto the granule surface (MacGregor, 1979).

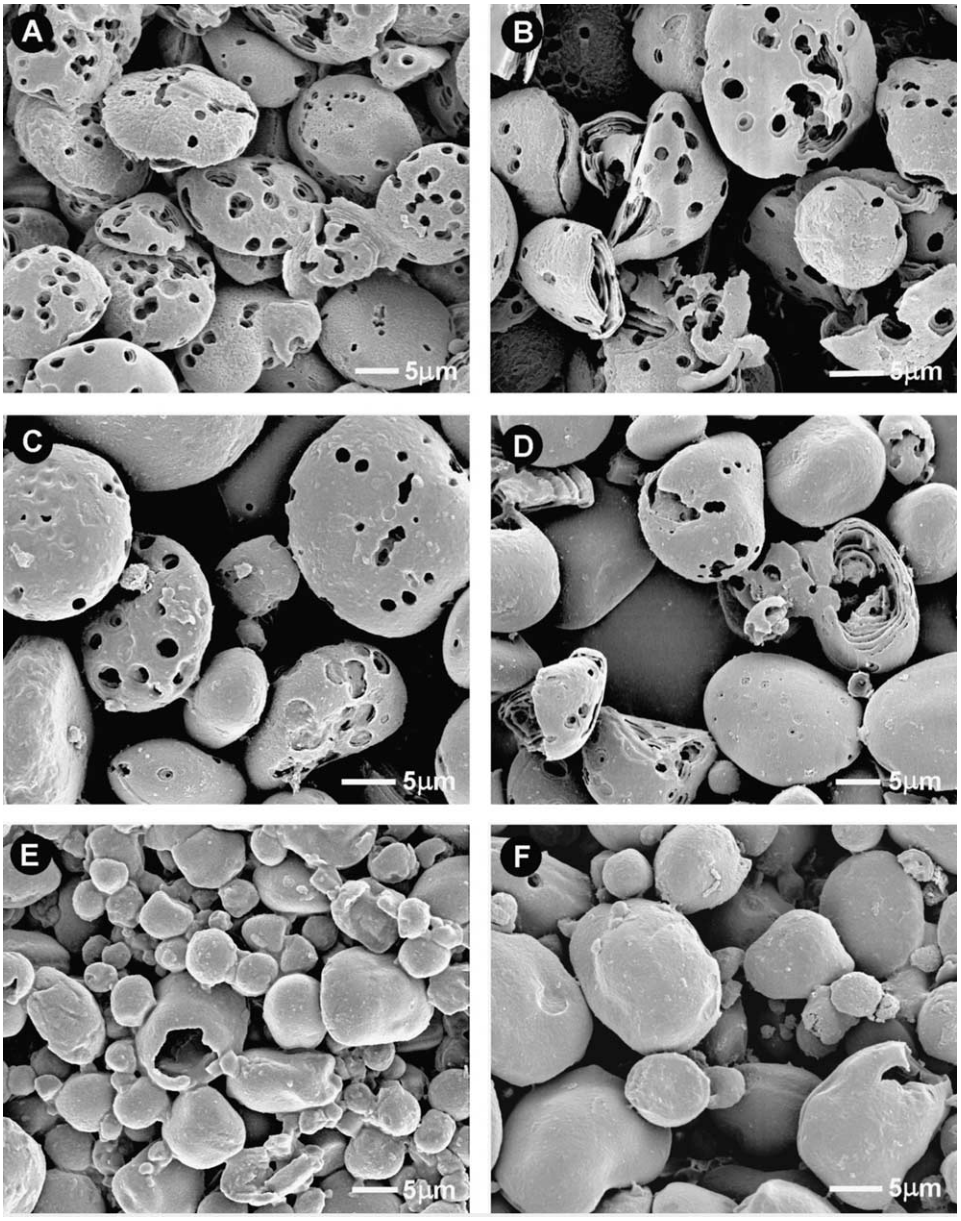

Fig. 4. Scanning electron micrographs of HB starch granules from waxy (CDC Alamo) (A & B), normal (CDC Dawn) (C & D), and high-amylose (SB 94893) types (E & F) hydrolyzed by PPA at 37 °C for 3 h (A, C & E) and 6h (B, D & F).

Pinholes/internal channels, which are naturally occurring features of many starch granules (Huber & BeMiller, 2000), may create preferential sites and specific areas on the granule surface and in the interior for enzyme diffusion and adsorption. Thus, the initial high hydrolysis rate shown by waxy HB starch (Fig. 2) may be partly due to the presence of pinholes and internal channels. Both normal and high-amylose HB starches have generally similar average granule size (6.7–6.8 μm in diameter) (Li et al., 2001a). However, high-amylose HB starch showed a higher rate of hydrolysis by amylases than normal starch at the initial stage (Fig. 2). This is perhaps due to the high proportion of small granules ($\leqslant$10 μm in diameter, 38% of total weight) in high-amylose HB starch compared to normal HB starch (11% in total weight) (Li et al., 2001a). Higher proportion of smaller granules lead to larger granule surface area to volume ratios. Also, microscopic observation (Valetudie et al., 1993) has shown that polyhedric shaped granules of tropical tuber starches (tannia, sweet potato and cassava) are hydrolyzed to a greater extent than spherical granules by α-amylases. Thus, granule morphology may influence the degree of susceptibility of starches towards to α-amylases.

The composition and concentration of hydrolysis products may also have an inhibitory effect on the rate of hydrolysis. Gradual reduction of the rate of hydrolysis may be partly caused by the inhibition, by oligosaccharides, of α-amylase activities (Colonna et al., 1992). Colonna et al. (1992) reported that, in the oligosaccharide–enzyme complex, the molar ratio between oligosaccharide and enzyme influences the adsorption equilibrium. The concentration increase, during amylolysis, in maltose and maltotriose, rather than in glucose or maltotetraose, influences the adsorption equilibrium. However, if amyloglucosidase is used in combination with α-amylases, amyloglucosidase can hydrolyze maltose and maltotriose into glucose and thereby continuously transform the reaction products of α-amylolysis (Colonna et al., 1992, Planchot et al., 1995).

3.3. Scanning electron microscopy

Scanning electron micrographs of the residues left after hydrolysis of waxy, normal and high-amylose HB starches by porcine pancreatic alpha-amylase (PPA) and amyloglucosidase (AAG) are presented in Figs. 3–6. The granule surfaces of all three HB starches showed roughened surfaces (Fig. 3) after 1 h hydrolysis by PPA. In waxy HB starch granules, many of the granules displayed highly perforated surfaces. The erosion areas were roughly circular to oval to elliptical ranging from 0.3 to 3 μm in diameter and penetrated through several

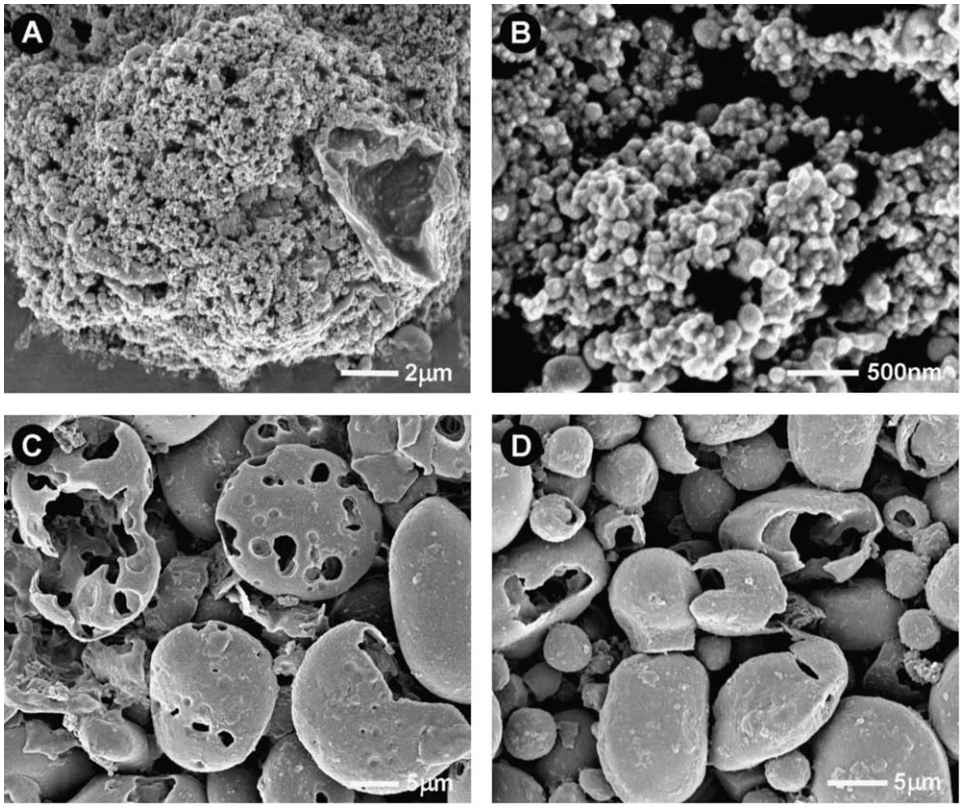

Fig. 5. Scanning electron micrographs of HB starch granules from waxy (CDC Alamo) (A & B), normal (CDC Dawn) (C), and high-amylose (SB 94893) types (D) hydrolyzed by PPA at 37 °C for 21 h.

layers of the granule into the interior (Fig. 3A). However, some granules were less eroded. Erosion areas with layered structure were less pronounced in granules of normal (Fig. 3C) and high-amylose (Fig. 3E) HB starches. The extent of erosion was higher in waxy HB starch. Some hollowed granules were present in high-amylose HB starches (Fig. 3 E). In all three starches, some granules were split open, revealing their internal layered structure (Fig. 3 B, D & F). This may have been due to structural weakness of the granule, resulting from hydrolysis along the equatorial groove plane, or due to mechanical damage during starch isolation. The internal layered structure showed, that in all three starches, the semi-crystalline growth rings gradually became closer (from the interior) to the surface (Fig. 3B, D & F). Furthermore, the size of central region of hydrolyzed HB starch granule followed the order: waxy $<$normal $<$high-amylose (Fig. 3 B, D & F). Split granules were more frequently observed in waxy HB starch than in normal and high-amylose HB starches. A similar erosion pattern has been reported in wheat starch (Evers & McDermott, 1970; Smith & Lineback, 1976) and legume starches (Bertoft, Manelius, & Qin, 1993; Tharanathan & Ramadas Bhat, 1988) hydrolyzed by alpha-amylase and glucoamylase. Kimura and Robyt (1995) also reported that the number of barley starch granules significantly increased (almost doubled) initially and then decreased during glucoamylase hydrolysis.

After 3 h of hydrolysis by PPA, erosion was more pronounced in the size and depth of erosion areas rather than increasing the number of erosion areas in all three HB starches (Fig. 4A, C & E). Some granules in waxy HB starch were fragmented (Fig. 4A). However, granule fragmentation was limited in normal (Fig. 4C) and high-amylose (Fig. 4E) HB starches. Most of the granules of normal and high-amylose HB starches retained their granular shape and showed a dense shell of varying thickness (Fig. 4C & E). After 6 h of hydrolysis, waxy HB starch was fragmented and deformed (Fig. 4B). However, in normal HB starches, many granules were deformed (Fig. 4D). The extent of deformation became more pronounced after 21 h of hydrolysis (Fig. 5C). Granules of high-amylose HB starch showed thin, granular shells with large hollows during 6 h (Fig. 4F) to 21 h of hydrolysis (Fig. 5D). However, in waxy HB starch, only a small amount of sponge-like residue was left after 21 h of hydrolysis (Fig. 5A). Under higher magnification (×25,000), the above residue appeared as clustered spherical particles (approximately 100nm in diameter), showing a layered structure (Fig. 5B). This suggests the presence of amylopectin crystals (cluster structure) in the semi-crystalline regions of the granule. The thin shell structure in hydrolyzed granules of normal and high-amylose HB starches suggests that the peripheral regions of these granules are resistant (due to a strong association between amylopectin molecules) to enzyme hydrolysis.

BAA hydrolysis showed a similar action pattern to that of PPA (micrographs not shown). However, AAG hydrolysis showed some different features, as depicted in Fig. 6. After 21 h of hydrolysis, wide, shallow and circular pits with internal layered structures were seen in the erosion areas of all three HB starches (Fig. 6A, B & C). The granules of waxy HB starch hydrolyzed by AAG were completely deformed and fragmented (Fig. 6 A), whereas, most granules in normal and high-amylose HB starches were less fragmented and deformed (Fig. 6B & C). Granules with thin shells and large hollows

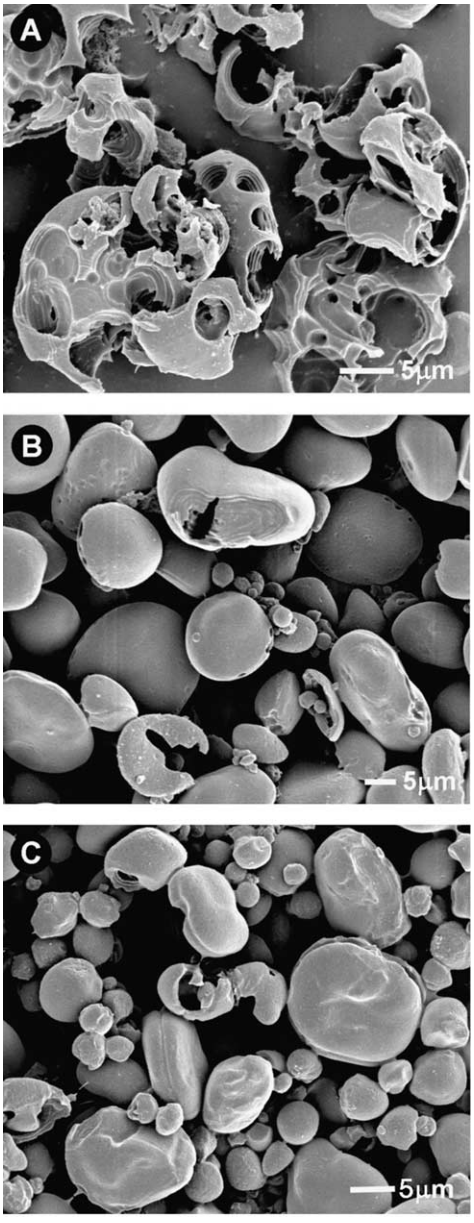

Fig. 6. Scanning electron micrographs of HB starch granules from waxy (CDC Alamo) (A), normal (CDC Dawn) (B), and high-amylose (SB 94893) types (C) hydrolyzed by AAG at 37 °C for 21 h.

were frequently observed in normal and high-amylose HB starches (Fig. 6B & C). Compared to PPA hydrolysis, AAG hydrolysis was confined to the tangential direction of the granule (Fig. 6).

3.4. Transmission electron microscopy

Transmission electron micrographs of the residues left after hydrolysis of waxy, normal and high-amylose HB starches by PPA and AAG are presented in Fig. 7 and Fig. 8, respectively. After 3 h of hydrolysis by PPA, hydrolyzed HB starch granules showed large cavities in their central regions (Fig. 7A, B & C), suggesting that rapid hydrolysis may have occurred in the central region. Channels in the peripheral regions of the granule were present in all three native HB starches (Fig. 7A, B & C). These were more pronounced in waxy HB starch (Fig. 7A). A number of studies (Leach and Schoch, 1961; Valetudie et al., 1993; Herbert et al., 1996) have shown that channels in the granule periphery provide a pathway for the entry of enzymes into the granule interior. These channels may be derived from inherent surface pores/internal channels in starch granules and enlarged by enzyme hydrolysis (Kimura and Robyt, 1995). Several outer layers of granules were left with a sharp saw-toothed structure on the edge of fragments

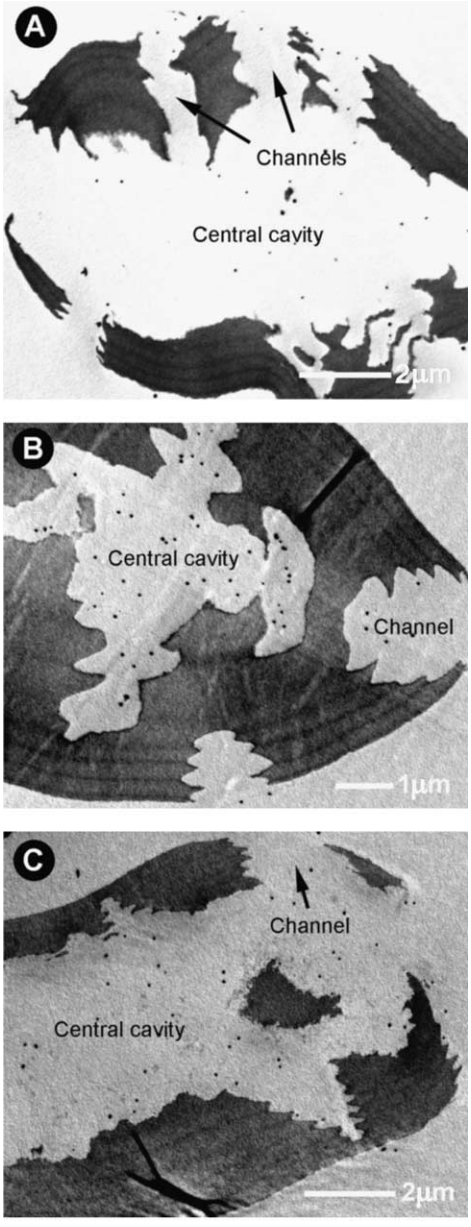

Fig. 7. Transmission electron micrographs of HB starch granules from waxy (CDC Alamo) (A), normal (CDC Dawn) (B), and high-amylose (SB 94893) types (C) hydrolyzed by PPA at 37 °C for 3 h.

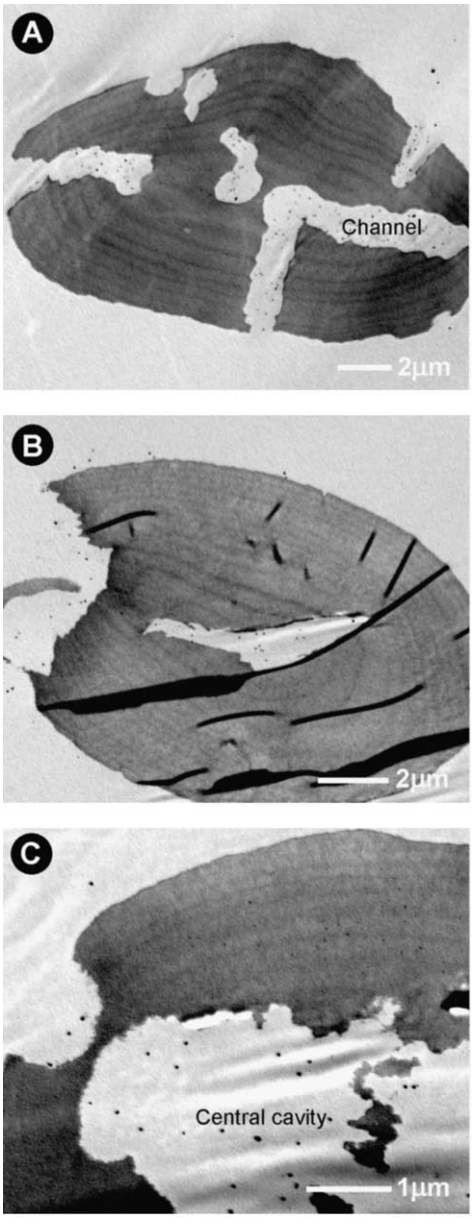

Fig. 8. Transmission electron micrographs of HB starch granules from waxy (CDC Alamo) (A), normal (CDC Dawn) (B), and high-amylose (SB 94893) types (C) hydrolyzed by AAG at 37 °C for 21 h.

(Fig. 7A, B & C). These observations suggests that a relatively faster hydrolysis (along the tangential direction of the granule) occurs in the amorphous region (dark rings) than in the semi-crystalline region (light rings). The hydrolysis patterns of HB starches by PPA and BAA are similar to those of other cereal starches (Gallant et al., 1972, 1992; Planchot et al., 1995), but differed with respect to some tuber starches (Gallant et al., 1992; Planchot et al., 1995; Valetudie et al., 1993). Valetudie et al. (1993) reported a difference, in enzymatic action on yam starch, between bacterial and pancreatic α-amylases. A sponge-like structure (numerous radial pores/canals) with bacterial α-amylase was formed in yam starch granules at the initial stage of hydrolysis, and fragmentation occurred at the final stage, whereas, a filamentous structure was retained, with resistant outer layer of granule, in the residue of starch hydrolyzed by pancreatic α-amylase.

After 21 h of hydrolysis of three HB starches by AAG, erosion areas were present in all three HB starch granules in the form of channels/pits, varying in width and depth and central cavities (Fig. 8A, B & C). Channels/pits were present to a greater extent in waxy (Fig. 8A) than in normal and high-amylose HB starches (Fig. 8B & C). Channels in waxy HB starch granules were vertical to the granule surfaces (Fig. 8A). Furthermore,

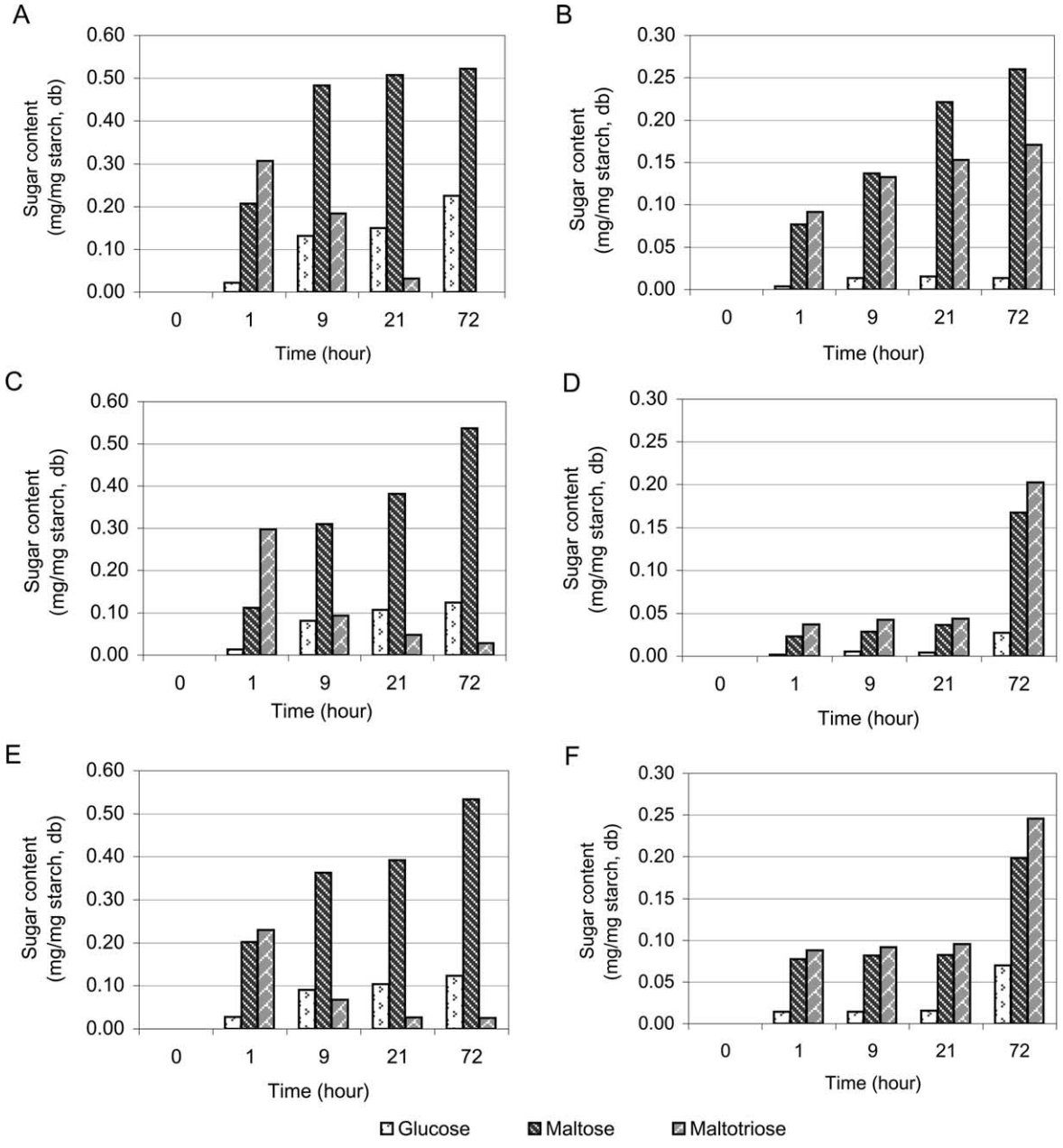

Fig. 9. The contents of glucose, maltose, and maltotriose produced during HB starch hydrolysis by PPA (A, C & E) and BAA (B, D &F). A and B: waxy (CDC Alamo); C and D: normal (CDC Dawn); E and F: high-amylose (SB 94893).

the saw-toothed edges observed during hydrolysis of HB starches by AAG were not as clearly visible (Fig. 8) as those of PPA and BAA. The above observations suggest that amorphous (dark rings) and crystalline (light rings) regions of all three HB starch granules were hydrolyzed in a similar fashion by AAG.

3.5. Hydrolysis products

The contents of glucose, maltose, and maltotriose in the hydrolysates of HB starches were determined by HPLC at different time periods of hydrolysis (Fig. 9). The composition and concentration of soluble products during hydrolysis differed with starch and enzyme types and with the duration of hydrolysis. At the onset of hydrolysis, no sugars were detected by HPLC. When PPA and BAA hydrolysis of three HB starches progressed, glucose, maltose, and maltotriose were produced, whereas, only glucose was detected during AAG hydrolysis. In all three starches, AAG hydrolysis (48 h) produced at least 2.8 times more glucose from waxy HB starch than from normal HB starch and 3.3 times more from in high-amylose HB starches. Hydrolysis (1 h) of three HB starches by PAA produced more maltotriose (0.23–0.31 mg/mg starch, db) than glucose (0.01–0.03 mg/mg starch, db) and maltose (0.11–0.21 mg/mg starch, db) (Fig. 9A, C & E). With extensive hydrolysis of three HB starches, the amount of maltose increased significantly, and glucose increased slightly, while maltotriose content decreased greatly. After 72 h of hydrolysis, the maltose content (0.52–0.54 mg/mg starch, db) was 2.3–4.3 times more than those of glucose (0.12–0.23 mg/mg starch, db) and maltotriose (0–0.03 mg/mg starch, db). These three sugars accounted for 68–75% of total starch from each starch source. During BAA hydrolysis (Fig. 9B, D & F), the contents of glucose, maltose, and maltotriose increased gradually, from all three HB starches. However, the amount of maltose increased faster than that of maltotriose from waxy HB starch, whereas, the content of maltose was lower than maltotriose from normal and high-amylose HB starches. After 72 h of hydrolysis, the contents of glucose, maltose, and maltotriose were in the range of 0.01–0.07, 0.17–0.26, 0.17–0.25 mg/mg starch (db), respectively. These three sugars accounted for 40–51% of total starch in each HB starch. In general, the concentration and composition of hydrolysis products (predominantly glucose, maltose, and maltotriose) depend on enzyme sources, origin and types of starches, and conditions of hydrolysis.

4. Conclusions

In-vitro hydrolysis patterns of HB starches were dependent on enzyme source and starch types. Transmission electron microscopy showed the pathway of starch hydrolysis by α-amylase and amyloglucosidase. The rate of hydrolysis, by PPA, BAA and AAG, of waxy HB starch, was higher than those of normal and high-amylose HB genotypes. The outer layers of normal and high-amylose HB starch granules were more resistant to enzyme hydrolysis. The concentration and composition of soluble sugars (glucose, maltose and maltotriose) in hydrolysates varied with enzyme source, starch types and the duration of hydrolysis. The study suggests that both morphological and ultrastructural features influence in-vitro hydrolysis of HB starches.

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References

Anger, H., Richter, M., Kettlitz, B., & Radosta, S. (1994). Hydrothermische Behandlung von Stärke in Gegenwart von α-Amylase Teil 1: Hydrolyse von Stärke mit α-Amylase in heterogener Phase. *Starch/Stärke*, *46*, 182–186.

Appelqvist, I. A. M., & Debet, M. R. M. (1997). Starch-biopolymer interactions—a review. *Food Review Inernational*, *13*, 163–224.

Bertoft, E., & Manelius, R. (1992). A method for the study of the enzymatic hydrolysis of starch granules. *Carbohydrate Research*, *227*, 269–283.

Bertoft, E., Manelius, R., & Qin, Z. (1993). Studies on the structure of pea starches. Part 1. Initial stages in α-amylolysis of granular smooth pea starch. *Starch/Stärke*, *45*, 215–220.

Bruner, R. L. (1964). Determination of reducing value. In R. L. Whistler (Ed.), *Methods of carbohydrate chemistry, Vol. 4* (pp. 67). New York: Academic Press.

Colonna, P., Buléon, A., & Lemarié, F. (1988). Action of *Bacillus subtilis* α-amylase on native wheat starch. *Biotechnology and Bioengineering*, *31*, 895–904.

Colonna, P., Leloup, V., & Buléon, A. (1992). Limiting factors of starch hydrolysis. *European Journal of Clinical Nutrition*, *42*, S17–S32.

Evers, A. D., & McDermott, E. E. (1970). Scanning electron microscopy of wheat starch II. Structure of granules modified by alpha-amylolysis—preliminary report. *Starch/Stärke*, *22*, 23–26.

Franco, C. M. L., & Ciacco, C. F. (1987). Studies on the susceptibility of granular cassava and corn starches to enzymatic attack. *Starch/Stärke*, *39*, 432–435.

French, D. (1984). Organization of starch granules. In R. L. Whistler, J. N. Miller, & E. F. Paschall (Eds.), *Starch: chemistry and technology* (pp. 183–248). New York: Academic Press.

Fuwa, H., Sugimoto, Y., & Takaya, T. (1979). Scanning electron-microscopy of starch granules, with or without amylase attack. *Carbohydrate Research*, *70*, 233–238.

Fuwa, H., Sugimoto, Y., Tanaka, M., & Glover, D. V. (1978). Susceptibility of various starch granules to amylases as seen by scanning electron microscope. *Starch/Stärke*, *30*, 186–191.

Gallant, D., Mercier, C., & Guilbot, A. (1972). Electron microscopy of starch granules modified by bacterial α-amylase. *Cereal Chemistry*, *49*, 354–365.

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

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

Garcia, V., Colonna, P., Bouchet, B., & Gallant, D. J. (1997). Structural changes of cassava starch granules after heating at intermediate water contents. *Starch/Stärke*, *49*, 171–179.

Gérard, C., Colonna, P., Buléon, A., & Planchot, V. (2001). Amylolysis of maize mutant starches. *Journal of the Science and Food Agriculture*, *81*, 1281–1287.

Helbert, W., Schülein, M., & Henrissat, B. (1996). Electron microscopic investigation of the diffusion of *bacillus licheniformis* α-amylase into corn starch granules. *International Journal of Biological Macromolecules*, *19*, 165–169.

Hoover, R., & Sosulski, F. W. (1991). Composition, structure, functionality, and chemical modification of legume starches: a review. *Canadian Journal of Physiology and Pharmacology*, *69*, 79–92.

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

Huber, K. C., & BeMiller, J. N. (2000). Channels of maize and sorghum starch granules. *Carbohydrate Polymers*, *41*, 269–276.

Kimura, A., & Robyt, J. F. (1995). Reaction of enzymes with starch granules: kinetics and products of the reaction with glucoamylase. *Carbohydrate Research*, *277*, 87–107.

Knutson, C. A., Khoo, U., Cluskey, J. E., & Inglett, G. E. (1982). Variation in enzyme digestibility and gelatinization behavior of corn starch granule fractions. *Cereal Chemistry*, *59*, 512–515.

Koba, Y., Saha, B. C., & Ueda, S. (1986). Adsorption on and digestion of raw starch by malt and bacterial alpha-amylases. *Journal of the Japanese Society of Starch Science*, *33*, 199–201.

Kurakake, M., Tachibana, Y., Masaki, K., & Komaki, T. (1996). Adosorption of alpha-amylase on heat-moisture treated starch. *Journal of Cereal Science*, *23*, 163–168.

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

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

Leach, H. W., & Schoch, T. J. (1961). Structure of the starch granule. II. Action of various amylase on granular starches. *Cereal Chemistry*, *38*, 34–46.

Li, J. H., Vasanthan, T., Hoover, R., & Rossnagel, B. (2003). Starch from hull-less barley. IV. Ultrastructure and distribution of granule-bound proteins. *Cereal Chemistry*, *80*, 524–532.

Li, J. H., Vasanthan, T., Rossnagel, B., & Hoover, R. (2001a). Starch from hull-less barley: I. Granule morphology, composition, and amylopectin structure. *Food Chemistry*, *74*, 395–405.

Li, J. H., Vasanthan, T., Rossnagel, B., & Hoover, R. (2001b). Starch from hull-less barley: II. Thermal, rheological and acid hydrolysis characteristics. *Food Chemistry*, *74*, 407–415.

MacGregor, A. W. (1979). Isolation of large and small granules of barley starch and a study of factors influencing the adsorption of barley malt α-amylase by these granules. *Cereal Chemistry*, *56*, 430–434.

MacGregor, A. W., & Ballance, D. L. (1980). Hydrolysis of large and small starch granules from normal and waxy barley cultivars by alpha-amylases from barley malt. *Cereal Chemistry*, *57*, 397–402.

Morrison, W. R. (1995). Starch lipids and how they relate to starch granule structure and functionality. *Cereal Foods World*, *40*, 437–446.

Nigam, P., & Singh, D. (1995). Enzyme and microbial systems involved in starch processing. *Enzyme and Microbiology Technology*, *17*, 770–778.

Planchot, V., Colonna, P., Gallant, D. J., & Bouchet, B. (1995). Extensive degradation of native starch granules by alpha-amylase from *Aspergillus fumigatus*. *Journal of Cereal Science*, *21*, 163–171.

Rasper, V., Perry, G., & Duitschaever, C. L. (1974). Functional properties of non-wheat flour substitutes in composite flours. II. Amylolytic susceptibility of non-wheat starches. *Canadian Institute of Food Science and Technology*, *7*, 166–174.

Rickard, J. E., Asaoka, M., & Blanshard, J. M. V. (1991). The physico-chemical properties of cassava starch. *Tropical Science*, *31*, 189–207.

Smith, J. S., & Lineback, D. R. (1976). Hydrolysis of native wheat and corn starch granules by glucoamylases from aspergillus niger and rhizopus niveus. *Starch/Stärke*, *28*, 243–249.

Tharanathan, R. N., & Ramadas Bhat, U. (1988). Scanning electron microscopy of chemically treated black gram (*Phaseolus mango*) and ragi (*Eleusine coracana*) starch granules. *Starch/Stärke*, *40*, 378–382.

Valetudie, J. C., Colonna, P., Bouchet, B., & Gallant, D. J. (1993). Hydrolysis of tropical tuber starches by bacterial and pancreatic α-amylases. *Starch/Stärke*, *45*, 270–276.

Vasanthan, T., & Bhatty, R. S. (1996). Physicochemical properties of small- and large-granule starches of waxy, regular, and high-amylose barleys. *Cereal Chemistry*, *73*, 199–207.

Vasanthan, T., Yeung, J., & Hoover, R. (2001). Dextrinization of starch in barley flours with thermostable alpha-amylase by extrusion cooking. *Starch/Stärke*, *53*, 616–622.

Wolf, B. W., Bauer, L. L., & Fahey, G. C. Jr (1999). Effects of chemical modification on in vitro rate and extent of food starch digestion: an attempt to discover a slowly digested starch. *Journal of Agricultural and Food Chemistry*, *47*, 4178–4183.