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Improved cold starch hydrolysis with urea addition and heat treatment at subgelatinization temperature

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ABSTRACT

We explore how the presence of urea can influence the kinetics of amylolysis, with a long-term objective of developing practical and energy efficient bioconversion protocols. In this study, triticale and corn starches were hydrolyzed by a granular starch hydrolyzing enzyme with or without addition of urea and a pre-heating treatment at subgelatinization temperature. Differential scanning calorimetry showed that the gelatinization parameters of triticale and corn starches were negatively correlated with the urea concentration in the starch suspension. Addition of urea did not significantly affect starch amylolysis by the granular starch hydrolyzing enzyme at 30 °C. However when pre-heating at a higher yet sub-gelatinization temperature (50 °C for triticale and 61 °C for corn, 5 °C below the onset of starch gelatinization) for 30 min, the presence of urea greatly facilitated the amylolysis of both tricticale and corn starches. Scanning electron microcopy revealed starch granule mophological changes to a porous structure in residual starch granules/fragments rich in resistant starch. This means that the amylolysis pattern in the presence of urea was fundamentally changed, and urea disrupts starch hydrogen bonds effectively with heating treatment at a sub-gelatinization temperature. This treatment combination increased both starch hydrolysis rate and extent. Since extra energy was not necessary to gelatinize starch, this method may benefit starch and bio-enthanol industries to reduce the costs of starch hydrolysis.

1. Introduction

In the newly emerging bio-ethanol industry, starchy cereal grains are the primary starting materials used in North America currently. Starch in cereal grains is converted into fermentable sugars through a common liquefaction and saccharification process by using thermal-stable α-amylase and amyloglucosidase. However, this practice is a challenging step that still remains costly due to high energy input and capital cost (Kwiatkowski, McAloon, Taylor, & Johnston, 2006) and low conversion efficiency due to the presence of the resistant portion of starch and the formation of non-fermentable constituents (Bhadra, Muthukumarappan, & Rosentrater, 2010; Galvez, 2005; Sharma et al., 2010). Native starch polymers need to be fully hydrated in order to be accessible to hydrolytic enzymes. This is normally achieved by heating starch slurries to 70–80 °C, representing a complex interdependent "unzipping" process of molecules known as gelatinization, where water penetrates even the tightly crystallized regions of starch granules. Starch polymers are hydrolyzed selectively as they are heated, with the easy-to-hydrate locations (i.e. amorphous regions) being hydrolyzed first, and leaving residual resistant fragments of starch aggregates that cannot be hydrated until 120 °C or even higher with steam injection (jet cooking). It is estimated that the energy demand is approximately 10–20% of the fuel value of ethanol produced (Robertson et al., 2006). Recent advances in simultaneous hydrolysis and fermentation using granular starch hydrolyzing enzymes under sub-gelatinization temperatures may lead to an innovation in ethanol production. This cold starch hydrolysis would provide many benefits, such as reduction of energy consumption, capital saving and improvement of fermentation yield. However, its low hydrolysis rate and/or incomplete hydrolysis due to the structural heterogeneity and crystalline nature of starch molecules are challenges to the adoption of this technology (Robertson et al., 2006). The bio-conversion efficiency of starch to glucose by certain hydrolytic enzymes greatly depends on starch source, structure (e.g. molecular weight and chain lengths of amylose and amylopectin, starch granule surface pores and internal channels, and granule crystalline structure) and composition (i.e. amylose/amylopectin ratio, granule associated protein and lipids, etc.). Therefore, understanding of the native starch granule structural features and factors that influence the kinetics of amylolysis become highly important for development of practical approaches to optimize starch amylolysis and energy efficient bioconversion.

Research has been undertaken on amylolysis of starches using α-amylase and amyloglucosidase after various pre-treatments and addition of natural or synthetic additives. The effects of heating

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at subgelatinization temperature (Chung, Lim, & Lim, 2006; Haska & Ohta, 1991, 1992; Shariffa, Karim, Fazilah, & Zaidul, 2009), hydrothermal treatment (annealing) (O'Brien & Wang, 2008), high pressure (Baks, Bruins, Matser, Janssen, & Boom, 2008; Hayashi & Hayashida, 1989; Selmi, Marion, Cornet, Douzals, & Gervais, 2000), extrusion (Baks, Kappen, Janssen, & Boom, 2008), addition of polysaccharides (Tester & Sommerville, 2003), addition of lipid (Crowe, Seligman, & Copeland, 2000; Cui & Oates, 1999; Lauro, Poutanen, & Forssell, 2000), addition of β-glucan (Faraj, 2004), and addition of phytic acid and polyphenols (Bjorck & Nyman, 1987) on the hydrolysis of pure starch have been described. Urea is an organic compound that is widely used as a popular solid nitrogen fertilizer, a constituent of cattle feeds and animal feedstock, a raw material for manufacture of resins and other products for various industrial applications, and also to be used in an eco-friendly way to reduce fuel emissions from power plants and diesel engines. Urea is a protein denaturant as it disrupts the noncovalent bonds (Kamoun, 1988) in the proteins. But it has little effect on the activity of α-amylases (Kubrak, Storey, Storey, & Lushchak, 2010; Shaw, Lin, Chen, & Chen, 1995). In aqueous suspension, urea assists starch gelatinization and solubilisation (Chiou, Fellows, Gilbert, & Fitzgerald, 2005; Hebeish, Elthalouth, & Elkashouti, 1981; Kuo & Wang, 2006). Urea is able to disrupt the intermolecular bonding rather than intramolecular hydrogen bonding in amylose and reduce the gel strength by decreasing the intermolecular network formation between water and amylose (McGrane, Mainwaring, Cornell, & Rix, 2004). However, there exist few, if any, reports on starch susceptibility to granular hydrolyzing enzyme in the presence of various concentrations of urea and effects of urea on the amylolysis kinetics of starch.

The objectives of this research were to: (1) investigate the effect of urea on the gelatinization properties of triticale and corn starches using differential scanning calorimetry; (2) study the effect of pre-heating treatment at sub-gelatinization (5 °C below the onset gelatinization temperature of individual starch) and urea addition at various concentrations on starch amylolysis; (3) characterize the amylolysis pattern of starch granules in the presence of urea using scanning electron microscopy and quantify the residual starches after enzymatic hydrolysis. The outcome of the research defines cost efficient approaches for quantitative starch conversion and better understanding of the amylolysis kinetics of starch as affected by urea addition.

2. Experimental

2.1. Materials

Triticale (× *Triticosecale* cv. Pronghorn) grain was supplied by the Field Crop Development Centre, Alberta Agriculture, Food and Rural Development (Lacombe, AB, Canada). Triticale grains were ground in a hammer mill (FitzMill D6, The Fitzpatrick Co., Elmhurst, IL) equipped with a 0.020 in. screen. Normal corn starch (Melojel) was provided by National Starch Food Innovation (Bridgewater, NJ). The granular starch hydrolyzing enzyme Stargen 002 (a mixture of α-amylase and glucoamylase, 570 GAU/g), was donated by Genencor International (Rochester, NY). All other chemicals and reagents used in this study were of analytical grade.

2.2. Starch isolation and purification

Triticale starch was isolated from whole grain flour at the Food Processing Development Centre (Leduc, AB, Canada) using a pilot-scale dough ball washing technique developed in our laboratory. The crude starch was further purified with mild alkali solution (0.05% NaOH, w/v) followed by neutralization (0.1 HCl) and thorough water washes in the laboratory. Pure, white starch was collected after scraping out of the brown layer on top of the residue in the centrifuge bottle. The purified starch had nitrogen content comparable to those of commercial cereal starches with a starch purity of 97% (dry basis).

2.3. Differential scanning calorimetry of purified starch

The thermal properties of triticale and corn starches (starch solid 30%) without or with addition of urea (0–40% of starch solids) was determined using a differential scanning calorimeter (DSC) (DSC Q100, TA Instruments-Waters LLC, New Castle, DE, USA). Thoroughly mixed sample (10–15 mg) was weighed into an aluminum DSC pan after the reaction mixture was prepared. The pan was hermetically sealed and equilibrated at ambient temperature for 0.5–1 h before loading to DSC cell. Indium was used as a calibration standard, and a sealed, empty aluminum pan was used as reference. Samples were heated from 5 °C to 140 °C at 10 °C/min. All samples were prepared twice and then run in DSC at least in duplicate. Gelatinization temperature parameters (onset, To; peak, Tp; conclusion, Tc) and endotherm enthalpy change (ΔH) were calculated using a thermal analysis software (Universal Analysis 2000, Version 4.5A, TA Instruments-Waters LLC).

2.4. Pre-heating treatment and starch amylolysis

Starch slurries (30% starch solid) were pre-treated in a shaking water bath at sub-gelatinization temperatures (50 °C for triticale and 61 °C for corn) for 30 min with addition of urea at concentration of 0–30% (dry starch basis) and then hydrolyzed by Stargen 002 (12 GAU/g) at 30 °C for 96 h. The concentration of reducing sugars in the supernatant of centrifuged samples was determined by the dinitrosalicylic acid (DNS) method (Bruner, 1964) and the degree of hydrolysis was expressed as the weight of glucose equivalents per 100 g dry starch. The residual starch after hydrolysis was collected for further characterization by scanning electron microscopy and compositional analysis.

2.5. Scanning electron microscopy

Starch samples were mounted on circular aluminum stubs with double-sided sticky tape, coated with gold to a thickness of 12 nm, and examined and photographed in a JEOL Model JSM 6301 FXV scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 5 kV.

2.6. Quantification of residual starch

A resistant starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) was used to measure the contents of resistant starch, solubilised (non-resistant) starch and total starch in the native starches and the residues of hydrolyzed starches.

2.7. Statistical analysis

All treatments and analyses were carried out in duplicate at least. Analysis of variance using the General Linear Model (GLM) procedure and correlation statistics was performed using SAS Statistical Software, Version 9.1.2 (SAS Institute Inc., Cary, NC, 2004). Multiple comparisons of the means were done using Tukey's test ($p < 0.05$). Mean values were reported in figures with error bars of ±standard deviation.

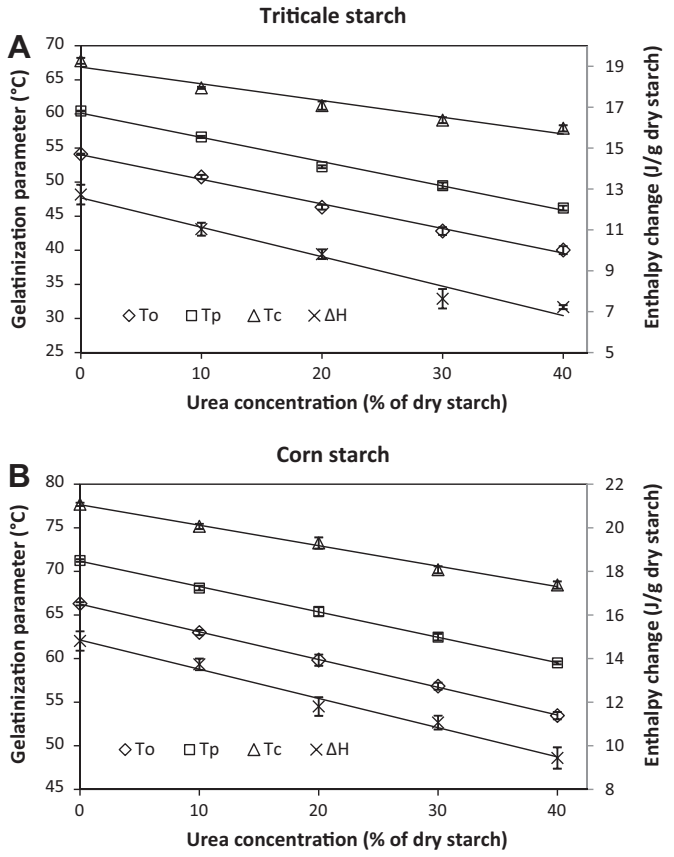

Fig. 1. Relationship between gelatinization parameters (gelatinization temperatures onset To, peak Tp, conclusion Tc and enthalpy change ΔH) and urea concentration of triticale (A) and corn (B) starches.

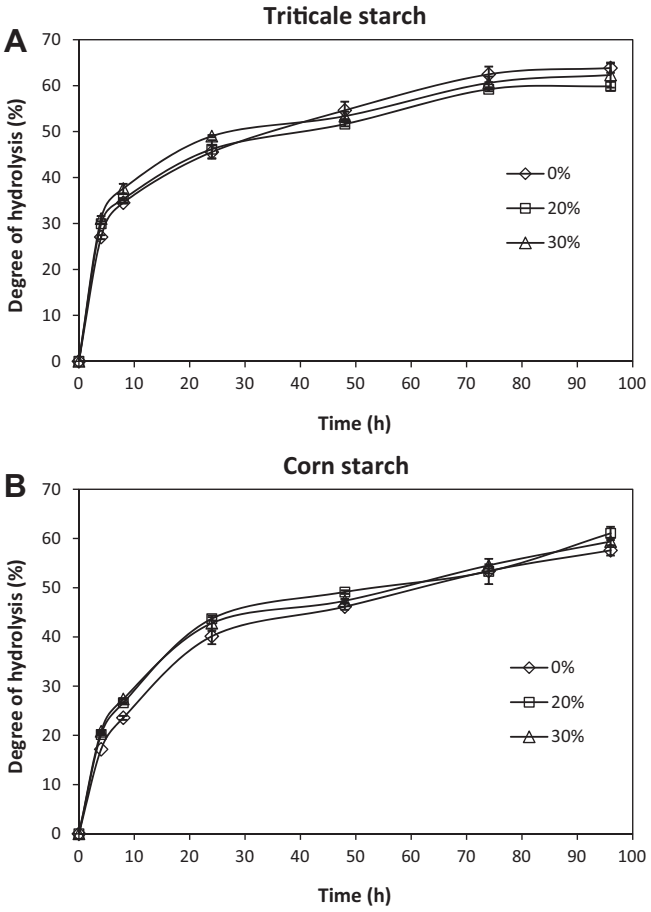

Fig. 2. Amylolysis of triticale (A) and corn (B) starches by granular starch hydrolyzing enzyme at 30 °C in the presence of urea (0–30% of starch solid).

3. Results and discussion

3.1. Starch gelatinization in the presence of urea

Gelatinization characteristics of triticale and corn starches in the presence of urea were performed by differential scanning calorimetry (DSC) and were plotted as a function of urea concentration in Fig. 1. The gelatinization temperatures (To, Tp and Tc) and enthalpy change (ΔH) of these two starches proportionally decreased with increase of urea concentration (up to 40% of starch solid) in starch suspensions, displaying a highly negative correlation with urea concentration ($R^2 > 0.96$, $p < 0.05$). Similar relationships were also found in waxy and regular rice starches (Kuo & Wang, 2006). DSC measures primarily the breakage of hydrogen bonds stabilizing double helices within starch granules, in which gelatinization enthalpy is a measure of the overall crystallinity of starch (quantity of crystallites) mainly reflecting the loss of double helical order while gelatinization temperature reflects crystallite quality (mainly double helix length and crystal uniformity) (Cooke & Gidley, 1992; Tester, 1997). Urea is an intermolecular hydrogen bond breaking agent, which is able to disrupt the intermolecular bonding in starch molecules (McGrane et al., 2004). Therefore, the decreases of gelatinization temperatures and enthalpy change indicated that urea partially disordered the crystalline structure of starch granules depending on urea concentration. The decreases of gelatinization temperatures and enthalpy change were more pronounced in triticale starch than in corn starch as evidenced by their slope values, indicating the diversities of crystalline structure and the degree of crystallinity of granules between triticale and corn starches. The decreases of gelatinization temperature and enthalpy change in the presence of urea could be associated with the increase of initial hydrolysis rate during amylolysis and final catalytic efficiency of hydrolyzing enzyme on starch. Tahir, Ellis, and Butterworth (2010) found that the gelatinization enthalpy is singnificantly correlated with the catalytic efficiency and amylolysis rate by porcine pancreatic α-amylase. The correlation of urea concentration with gelatinization enthalpy of stach could provide useful information for predicting amylolysis rate and extent at sub-gelatinization temperatures.

3.2. Starch susceptibility to granular starch hydrolyzing enzyme in the presence of urea

As shown in Fig. 2, increasing urea concentration (up to 30% of starch solid) did not significantly affect the degree of hydrolysis of both starches, suggesting that urea did not break the hydrogen bonds within starch granules at the hydrolysis temperature. The data also showed that granular starch hydrolyzing enzyme was resistant to urea denaturation at the concentration range used. There was no interaction between starch and urea and between urea and enzyme at room temperature. Kubrak et al. (2010) reported that the activity of pure *Bacillus* sp. α-amylase is quite stable in the presence of urea at concentration of up to 4 mol/L. α-Amylase from *Thermus* sp. is also not sensitive to even 8 mol/L of urea (Shaw et al., 1995).

3.3. Starch susceptibility to granular starch hydrolyzing enzyme with pre-heating treatment at sub-gelatinization temperature

Pre-heating of starch suspension at sub-gelatinization temperature (50 °C for triticale starch and 61 °C for corn starch, which was

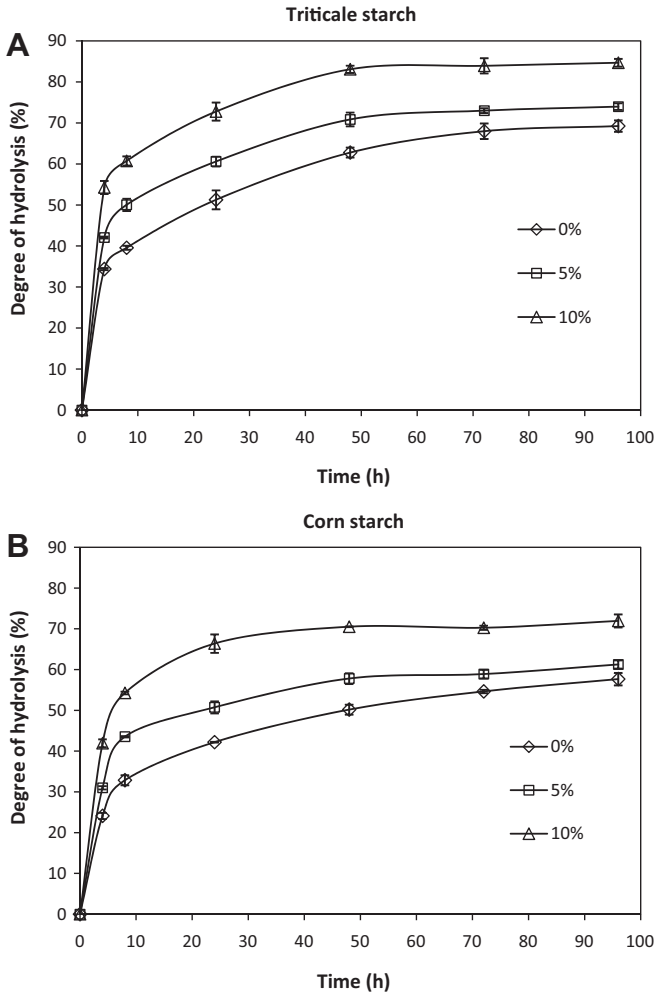

Fig. 3. Amylolysis of triticale (A) and corn (B) starches by granular starch hydrolyzing enzyme at 30 °C in the presence of urea (0–10% of starch solid) with pre-heating treatment at sub-gelatinization temperature (50 °C for triticale starch and 61 °C for corn starch for 30 min).

set as 5 °C below the onset gelatinization temperature determined by DSC) for 30 min prior to amylolysis effectively improved the starch amylolysis by increasing initial hydrolysis rate and extent (Fig. 3). Compared to the amylolysis without pre-heating treatment (Fig. 2), the amylolysis with pre-heating resulted in an increase of degree of starch hydrolysis by approximately 5–9% at 8 h hydrolysis and 4–8% at 48 h hydrolysis (Fig. 3) for triticale and corn starches. This result is consistent with amylolysis of tuber and root as well as sago starches using granular starch hydrolyzing enzymes with heating treatment at subgelatinization temperature (Haska & Ohta, 1991, 1992; Shariffa et al., 2009). Shariffa et al. (2009) reported that pre-heated tapioca and sweet potato starches at 60 °C for 30 min enhanced the degree of hydrolysis by 14% and 7% after 24 h hydrolysis, respectively, compared to those starches without pre-heating treatment. Haska and Ohta (1991,1992) also found that pre-heating at temperature below gelatinization temperature and lower pH greatly increased the ability of enzyme to hydrolyze sago starch. Pre-heating at sub-gelatinization temperature may cause partial disruption of starch structure by slight swelling of the amorphous region and expansion of the surface pinholes, internal channels and cavities of native starch granules, making starch granules more susceptible to enzyme access (Shariffa et al., 2009). However, even partial gelatinization of rice starch by heating (at 60, 65, and 70 °C for 5 min) increased starch hydrolysis rate at the initial stage but the maximum hydrolysis extent reached was still 5% lower than that of fully gelatinized starch (Chung et al., 2006).

3.4. Starch susceptibility to granular starch hydrolyzing enzyme in the presence of urea with pre-heating treatment at sub-gelatinization temperature

When starches were hydrolyzed by granular starch hydrolyzing enzyme after pre-heating in the presence of urea at sub-gelatinization temperature (50 °C for triticale starch and 61 °C for corn starch) for 30 min, increasing urea concentration to 10% (starch solid basis) remarkably increased the hydrolysis rate at initial stage and hydrolysis extent (Fig. 3). During the time course of amylolysis, a relatively rapid hydrolysis at first 8 h was followed by a progressively decreased hydrolysis rate thereafter. The

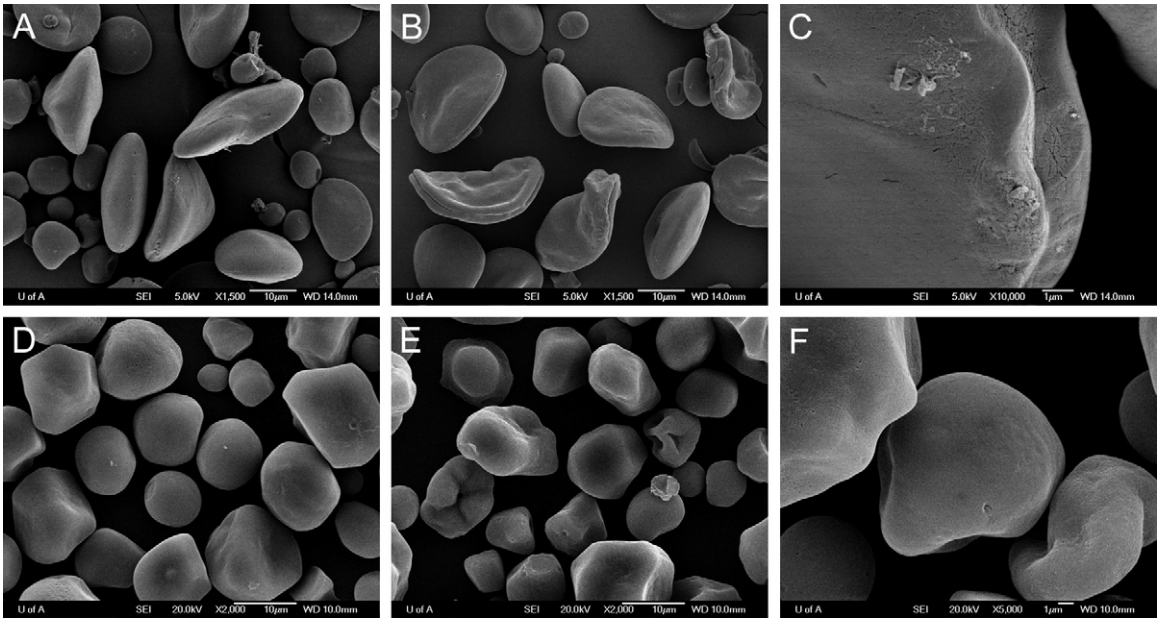

Fig. 4. Scanning electron micrographs of triticale (A, B and C) and corn (D, E and F) starch granules heated at sub-gelatinization temperature (50 °C for triticale and 61 °C for corn starches) for 30 min in the presence of 0% (A and D) and 5% (B, C, E, and F) of urea (starch solid basis).

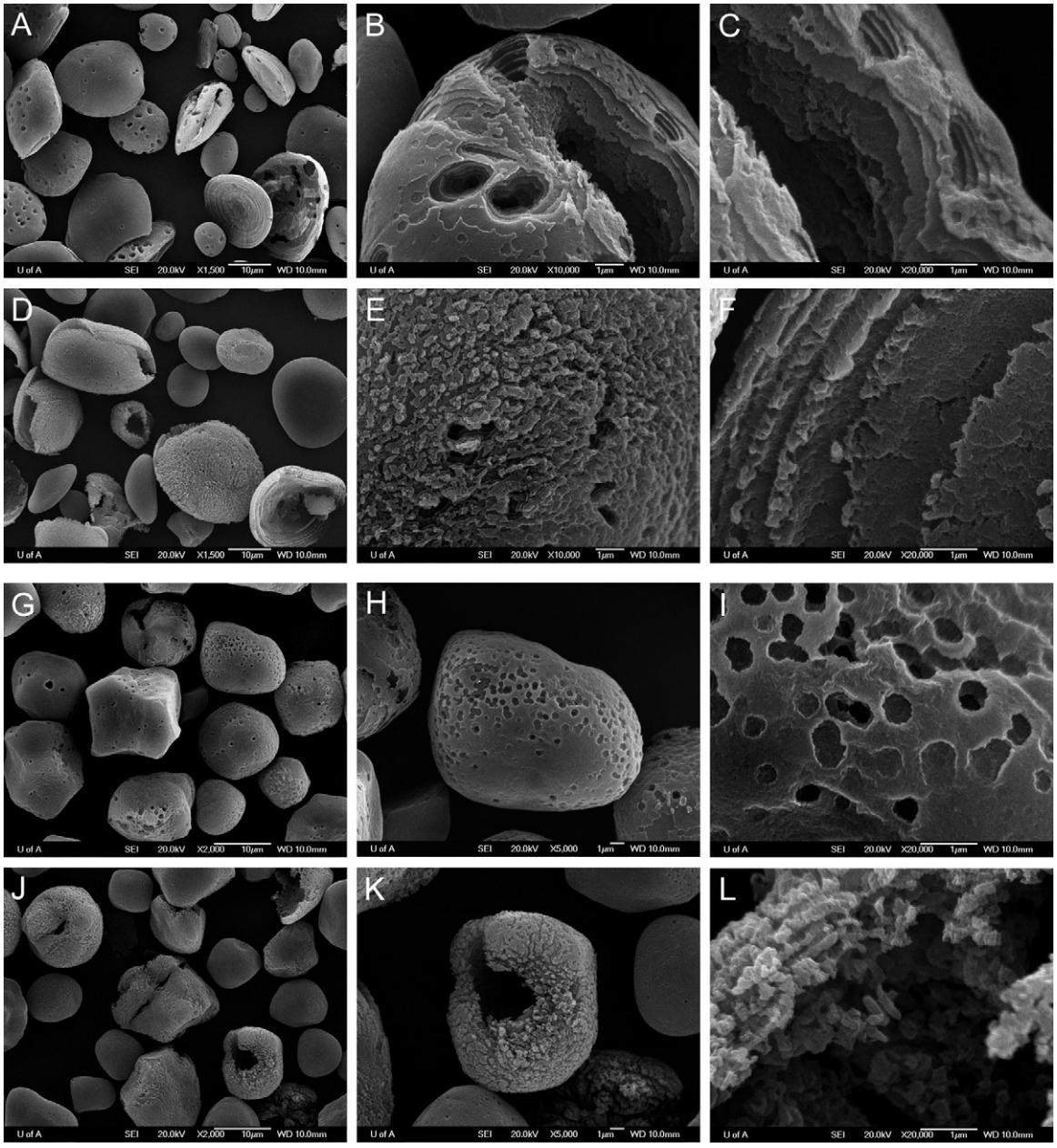

Fig. 5. Scanning electron micrographs of triticale (A-F) and corn (G–L) starch granules hydrolyzed by granular starch hydrolyzing enzyme at 30 °C for 24 h (A, B, C, G, H and I) and 8 h (D, E, F, J, K and L) with pre-heating at sub-gelatinization temperature (50 °C for triticale and 61 °C for corn starches) for 30 min in the presence of 0% (A–C and G–I) and 5% (D–F and J–L) of urea (starch solid basis). The degree of hydrolysis was 56% for triticale starch and 48% for corn starch.

hydrolysis extent reached the maximum after 48 h with both pre-heated starches in the presence of 10% urea (starch solid basis), whereas the degree of hydrolysis was still gradually increased at 96 h in the pre-heated starches in the absence of urea, indicating that addition of urea shortened the amylolysis course. After 96 h hydrolysis, pre-heated triticale and corn starches in the presence of 10% urea (starch solid basis) were hydrolyzed to an extent approximately 15% more than those starches in the absence of urea (85% vs. 69% for triticale starch and 72% vs. 58% for corn starch). The study indicated that urea facilitated the disruption of hydrogen bonds of starch molecules during heating. The amount of residual starch after hydrolysis was negatively proportional to urea concentration ($y = -6.9111x + 42.356$, $R^2 = 0.9339$ for triticale starch and $y = -4.4311x + 50.911$, $R^2 = 0.9828$, $p < 0.05$), indicating that the amount of hydrogen bond breaking depends on the concentration of urea in starch suspension.

3.5. Morphological changes of starch granules during amylolysis in the presence of urea

Scanning electron microscopy has revealed that native triticale starch granules were round, oval, disk-like lenticular or somewhat irregular in shape, and exhibited a bimodal size distribution, whereas corn starch showed polyhedric granules with numerous large, individual pores on the granule surfaces (Naguleswaran, Li, Vasanthan, & Bressler, 2011). Both starch granule surfaces appeared to be relatively smooth with modest furrows and shallow depressions on some. Under high magnification, oval-shaped pores along equatorial grooves and some aggregates of small pores with the appearance of slit-like cracks were clearly observed on the large granule surfaces of triticale starches.

As shown in Fig. 4, pre-heating treatment of starch suspensions at sub-gelatinization temperature caused slight twisting of

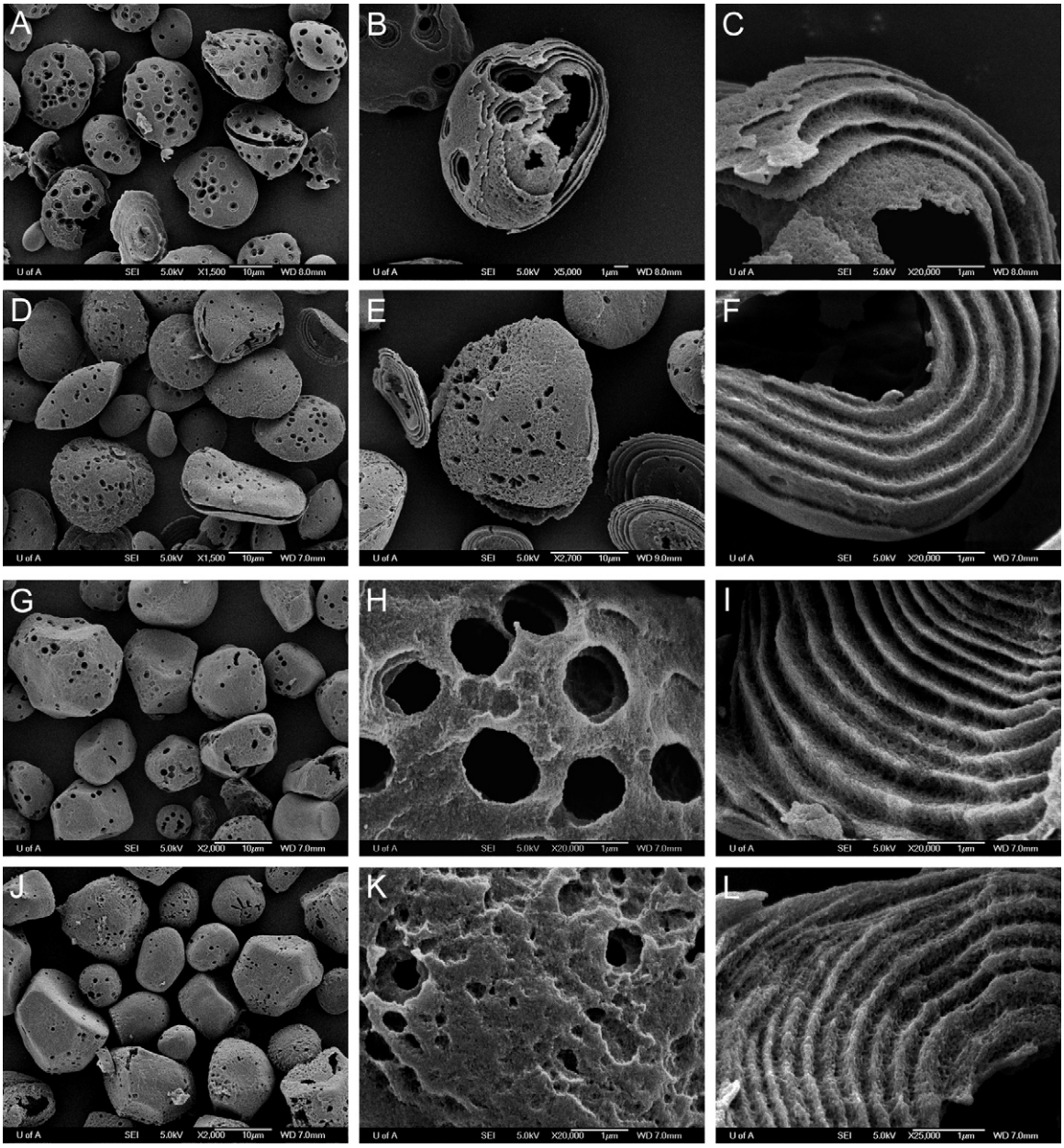

Fig. 6. Scanning electron micrographs of triticale (A–F) and corn (G–L) starch granules hydrolyzed by granular starch hydrolyzing enzyme at 30 °C for 96 h with pre-heating at sub-gelatinization temperature (50 °C for triticale and 61 °C for corn starches) for 30 min in the presence of 0% (A–C and G–I) and 5% (D–F and J–L) of urea (starch solid basis).

lenticular large granules in triticale starch (Fig. 4A) and appearance of ridges on granule surfaces of both starch granules (Fig. 4A and D). The shape change was much more pronounced with addition of 5% urea (starch solid basis) (Fig. 4B and E), indicating that the presence of urea induced more uneven granular swelling in the radial and tangential directions at the sub-gelatinization temperature. The uneven swelling was attributed to the heterogeneous molecular organization of starch granules. Randomly distributed pinholes were more visible on the surfaces of large starch granules from both triticale and corn (Fig. 4A–F) and small crevices/fissures appeared in the ridge areas of triticale starch granules (Fig. 4C). These structurally weakened regions due to thermal treatment along with surface pores could be starting points for enzyme erosion/attack and towards the center of the granule as revealed by atomic force microscopy (AFM) and SEM (Fannon, Hauber, & Bemiller, 1992; Thomson, Miles, Ring, Shewry, & Tatham, 1994). The morphological changes of starch granules in the present study suggested that urea may weaken and/or disrupt the hydrogen bonds mostly in the amorphous and/or non-crystalline regions of the starch granule, thus exposing more surface area for enzyme to adsorb and access the glycosidic linkages. The surface area of the granule has been correlated with the catalytic efficiency of amylase and the initial rate of hydrolysis (Kim, Kong, Kim, & Lee, 2008; Kong, Kim, Kim, & Kim, 2003; Tahir et al., 2010; Tester, Qi, & Karkalas, 2006).

Fig. 5 shows the morphological changes of starch granules at similar degree of hydrolysis (56% for triticale and 48% for corn starches). As shown in Fig. 5A, B, G, and H, after 24 h hydrolysis of triticale and corn starches in the absence of urea (0%), many of the hydrolyzed starch granules displayed highly perforated erosion pits with layered structure on the granule surfaces, even though individual granules were unevenly hydrolyzed. It appeared that the pits were initiated from enzymatic erosion of pores and weak regions on the granule surface by enlargement of the pinhole size, creation of new pores, and penetration into granule interior. The

AFM study indicated that enzyme action deepens existing pinholes and also creates new pores on the granule surface (Sujka & Jamroz, 2009). Numerous pits deepened through several layers of the granule into the interior (Fig. 5B, C, H and I), showing a typical amylolysis pattern (exocorrosion and endocorrosion) as reported in common cereal starches (Oates, 1997). Some granules in triticale starch were split open along the equatorial groove plane, revealing their internal layered structure (Fig. 5C), while a few hollowed granules were present in corn starch (Fig. 5G). However, after 8 h hydrolysis of triticale and corn starches in the presence of urea (5%, starch solid basis), most of the hydrolyzed starch granules became spongy, forming porous surfaces over the entire granule with many split granule halves, fragments and hollowed granules (Fig. 5D, E, J and K) and fragmented internal ring structure (Fig. 5F and L).

After 96 h hydrolysis without the presence of urea, starch granules were much more eroded as evidenced by the apprearce of enlarged holes on granule surfaces, and deformed granule fragments in both triticale and corn starches (Fig. 6A and G). Many granules were still in granular shape with a few thin layers and a large hollow (Fig. 6A, B, C, G, H and I). However, when urea was present during hydrolysis, the residual starch granules showed some different structural features. The granule surface was extensivly eroded and became more rough and porous with numerous craters of varying size and depth (Fig. 6D, E, J and K). The size of enlarged pores (Fig. 6D, E, J and K) was smaller than those in the absence of urea (Fig. 6A, B, G and H). Also, the surfaces of internal granule fragments as visible alternative rings were more fibrillar in the radial direction (Fig. 6F and L) rather than relativly smooth (Fig. 6C and I). The SEM study confirmed that the presence of urea with pre-heating treatment totally altered the amylolysis pattern of both triticale and corn starches.

3.6. Quatification of residual starch after amylolysis

The total (non-resistant/solublilised and resistant) amount of residual starches after 96 h amylolysis were 35.1% for triticale (Fig. 7A) and 43.0% for corn (Fig. 7B) starches without pre-heating treatment and addition of urea. Pre-heating treatment reduced the amount of residual starch to 23.1% in triticale (Fig. 7A) and 40.4% in corn (Fig. 7 B) starches after 96 h hydrolysis. The presence of urea in starch suspension at the concentration of 10% (starch solid basis) further reduced the residual starch to 14.1% in triticale (Fig. 7A) and 30.6% in corn (Fig. 7B) starches. The contents of resistant starch in total residual starches were increased singnificantly from 0.5% to 15.7% and from 1.4% to 2.1% in triticale and corn starches, respectively, with increasing concentration of urea to 10% of starch solids. This should be attributed to the structural difference between two starches, especially the structure of crytalline regions in starch granules. X-ray diffraction and microscopy studies have demonstrated that enzymatic erosion preferentially occurred in the amorphous regions of the granules (Franco, Preto, Ciacco, & Tavares, 1988; Shariffa et al., 2009). Thus, the amorphous region was hydrolyzed faster than the crystalline region, shown as a saw-tooth structure as revealed by SEM in this study and by SEM and TEM in previous studies (Helbert, Schulein, & Henrissat, 1996; Li, Vasanthan, Hoover, & Rossnagel, 2004; Planchot, Colonna, Gallant, & Bouchet, 1995). When most of the starch molecules in the amorphous regions in granules were hydrolyzed in triticale, the more resistant part of starch in the crystalline region was left as residual starch as shown by the acccumulation of resistant starch. The proportion of resistant starch was influenced by both enzyme and starch sources. Sharma et al. (2010) reported that liquefaction at higher temperature promotes amylolysis by leaving low amounts of resistant starch and total residual starch, resulting in higher ethanol yield after fermentation.

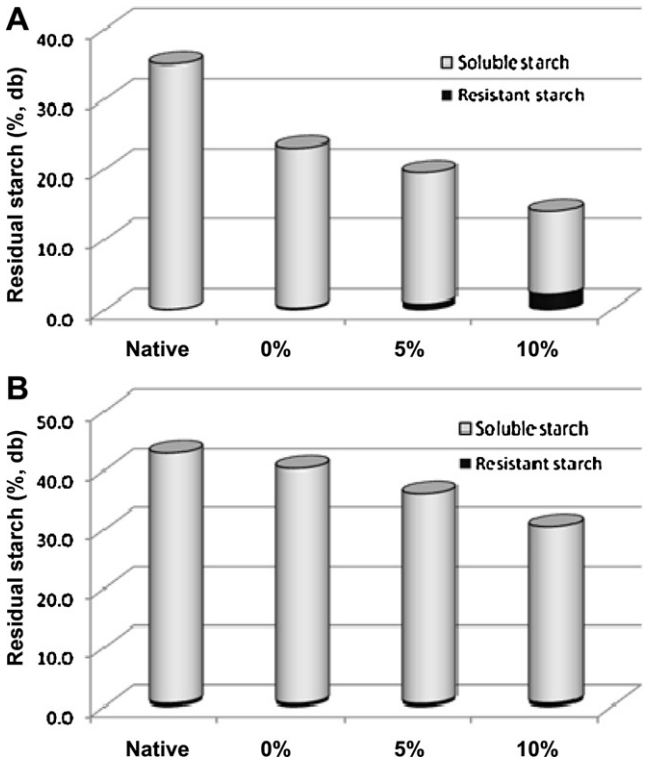

Fig. 7. Residual starch after amylolysis of triticale (A) and corn (B) starches by granular starch hydrolyzing enzyme at 30 °C for 96 h. Native represents starch amylolysis by granular hydrolyzing enzyme without pre-heating treatment. 0%, 5%, and 10% represent starch amylolysis with pre-heating treatment (at 50 °C and 61 °C for 30 min for triticale and corn starches, respectively) in the presence of urea at the concentration of 0, 5, 10% of starch solid.

4. Conclusions

Gelatinization temperature and enthalpy change of triticale and corn starches were negatively correlated with the urea concentration. Addition of urea to starch suspensions did not significantly affect starch amylolysis using granular starch hydrolyzing enzyme at 30 °C, but with pre-heating at a subgelatinization temperature (5 °C below the onset starch gelatinization temperature) for 30 min, the presence of urea in starch suspension greatly facilitated the amylolysis of both tricticale and corn starches. The mophological changes of starch granules revealed by SEM indicated that the amylolysis pattern in the presence of urea was totally changed, resulting in a porous structure in residual starch granules and fragments. This study indicates that urea breaks hydrogen bonds in starch molecules effectively at a sub-gelatinization temperature. The combination of urea addition and pre-heating treatment at this sub-gelatinization temperature greatly improves starch amylolysis by increasing the hydrolysis rate and extent, reducing hydrolysis time, bypassing the need for extra energy to gelatinize starch, without causing viscosity difficulties associated with gelatinization. This simple and practical approach can be adapted to the starch and bioenthanol industries to lower energy costs by increasing the starch conversion efficiency.

Acknowledgments

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