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Comparison of the physicochemical properties of barley starches after partial α-amylolysis and acid/alcohol hydrolysis

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Abstract

Zero amylose waxy (CDC Alamo), normal (Falcon), and high amylose (CDC 92-55-06-48) hull-less barley starches were isolated and subjected to α-amylase (*Bacillus licheniformis*) hydrolysis (24 and 48 h; 50 and 500 U/g) and acid hydrolysis (2% HCl, 10 and 96 h) in different alcohol media (methanol, ethanol, and 1-butanol) to determine the molecular characteristics of the starch material remaining in the granules after treatments. The greatest yield of α-amylase-treated starches was obtained for the high amylose (79.9–86.6% w/w) followed by normal (61.1–76.1% w/w), and zero amylose waxy starch (44.2–64.5% w/w). Very little solubilization of starches occurred during acid/alcohol hydrolysis, and very high yields of treated starches were obtained (91–99%). Relatively small decreases in the average molecular weights (M_w) of starch components were observed after α-amylolysis; the extensive hydrolysis of the zero amylose starch (56% solubilization) caused only a threefold decrease in the M_w of amylopectin. The M_w of starch fractions after acid/alcohol hydrolysis was affected by the hydrolysis time and alcohol but not by the amount of amylose in the native starches; the M_w and the mode of distribution of various M_w species can be tailored to specific needs by choosing appropriate hydrolysis conditions. The partially enzyme-hydrolyzed normal and high amylose starches formed stronger gels (40% w/w) than their native counterparts, but waxy starch showed no evidence of network development during storage (20 h, 5 °C). The partially acid-hydrolyzed high amylose starch (2% HCl/MeOH, 10 h) showed significantly higher values of G' compared to the native sample upon storage, whereas the partially hydrolyzed normal starch exhibited the G' values similar to its native counterpart. Both normal and high amylose starches, hydrolyzed for 96 h, exhibited a rapid rise in G' upon cooling but very little network development thereafter.

Keywords: Barley starches; Acid/alcohol hydrolysis; α-Amylolysis; Molecular weight; Viscoelasticity

1. Introduction

Barley is one of the most widely cultivated cereal crops that can provide valuable nutrients required by humans and domestic animals. The interest in barley as a food component has increased because of the potential health benefits of many constituents of this grain: dietary fibre, phenolic compounds, and vitamins (Jadhav, Lutz, Ghorpade, & Salunkhe, 1998). Starch is the major component of barley grain and, although it has not been studied to the same extent as corn, wheat or rice starch, it has recently gained a lot of interest due to its potential to replace other common starches in food and industrial applications. The molecular structure and physicochemical properties of isolated barley starches with amylose content ranging from 0% to 40% have been described in recent reports (Li, Vasanthan, Rossnagel, & Hoover, 2001a, 2001b; Song & Jane, 2000; Suh, Verhoeven, Denyer, & Jane, 2004; Takeda, Takeda, Mizukami, & Hanashiro, 1999; Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2001; Tang, Watanabe, & Mitsunaga, 2002; Tang, Mitsunaga, & Kawamura, 2004; Yoshimoto, Takenouchi, & Takeda, 2002; You & Izydorczyk, 2002; Zheng, Han, & Bhatty, 1998).

Enzymic and acid hydrolyses have been used traditionally to modify native starches and to create products with

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altered solubility, viscosity, and/or gelation properties that find broad applications in food, paper, textile, and other industries (Atichokudomchai, Shobsngob, Chinachoti, & Varavinit, 2001; Hoover, 2000; Virtanen, Autio, Suortti, & Poutanen, 1993; Wang & Wang, 2001). α-Amylases can hydrolyze both the soluble starch polymers as well as the intact starch granules (Gallant, Mercier, & Guilbot, 1972; Helbert, Schulein, & Henrissat, 1993; MacGregor & Ballance, 1980; Planchot, Colonna, Gallant, & Bouchet, 1995). Generally, the source of α-amylases, the botanical origin of starch, and the ultrastructural features of granule organization (inter-chain associations, type and degree of cystallinity, and amylose–lipid interactions) influence the hydrolysis kinetics (Banks & Greenwood, 1975; Colonna, Leloup, & Buleon, 1992; Gallant, Bouchet, Buleon, & Perez, 1992; Gerard, Colonna, Buleon, & Planchot, 2001; Lauro, Suortti, Autio, Linko, & Poutanen, 1993; Leach & Schoch, 1961; Planchot et al., 1995). It has been accepted that waxy starches are more susceptible to enzyme hydrolysis than high amylose starches (Cone & Wolters, 1990; Leach & Schoch, 1961; MacGregor & Ballance, 1980). The starch crystallinity type affects the efficiency of granule solubilization, with the B-type crystals being less susceptible to enzyme hydrolysis than the A-type (Jane, Wong, & McPherson, 1997; Planchot, Colonna, & Buleon, 1997). Also, the isolated and purified small barley starch granules were found to have a greater susceptibility to cereal α-amylases than large ones, which was attributed to the larger surface area of small granules when compared to large ones on an equal weight basis (Bertoft & Kulp, 1986; MacGregor & Ballance, 1980; MacGregor & Morgan, 1986).

The differences in the rate and degree of acid hydrolysis of granular starch have also been attributed to differences in granular size, extent and type of chain interactions within the granule (i.e. degree and type of crystallinity) and starch composition (amylose content, degree of phosphorylation) (Gerard, Colonna, Buleon, & Planchot, 2002; Hoover, 2000; Jacobs, Eerlingen, Rouseu, Colonna, & Delcour, 1998; Jane et al., 1997; Li, Vasanthan, Rossnagel, & Hoover, 2001b). For example, small granules in barley starches were reported to have a higher rate of acid hydrolysis than large ones, presumably due to their larger surface area per unit weight (Vasanthan & Bhatty, 1996). Also, high amylose barley starch was reported to be less susceptible to acid hydrolysis than normal and waxy barley starches (Li et al., 2001b). It was suggested that the highly compact amorphous regions in high amylose starch granules, resulting from extensive inter-chain associations of amylose polymers, prevent penetration of acid into the granules (Li et al., 2001b; Vasanthan & Bhatty, 1996).

The most traditional acid hydrolysis procedures used for modification of starch include those reported by Nageli (1874), Lintner (1886). The Nageli procedure involves treatment of native starch with 15% sulfuric acid for one month at room temperature and produces hydrolyzates that are readily soluble in hot water and contain a mixture of low molecular weight (M_w) linear and branched dextrins (DP: 25–30). The Lintner procedure involves hydrolysis of native granules in 7.5% (w/v) HCl for one week and results in a relatively high molecular weight product. In industry, the acid-modified starches are prepared by treating starch slurries with dilute HCl or H_2SO_4 at 25–55 °C for various periods of time. The molecular weight and viscosity of acid-modified starches vary with the conditions used during modification, however, their yield decreases consistently with increasing acid concentration and hydrolysis time. Robyt and co-workers (Ma & Robyt, 1987; Robyt, Choe, Hahn, & Fuchs, 1996) and Lin and co-workers (Lin, Lee, & Chang, 2003; Lin, Lii, & Chang, 2005) showed that the amount of low molecular weight dextrins can be minimized and, therefore, a high yield (88–100%) of water soluble starch granules can be obtained in a relatively short time (1–72 h) by conducting acid hydrolysis of potato and waxy maize starch in various alcohols. Acid hydrolysis in alcohols results not only in a high recovery but also uses less amount of acid than traditional acid hydrolysis (Chang, Lin, & Chang, 2006). The degree of polymerization (DP) and molecular weight distribution of starch polymers inside the granules can be controlled by varying the type and concentration of alcohol, concentration of acid, and the temperature of hydrolysis (Chun, Lim, Takeda, & Shoki, 1997; Lin et al., 2005; Ma & Robyt, 1987).

Despite several published studies on hydrolysis of barley starch granules by α-amylase (Bertoft & Kulp, 1986; Bertoft & Avall, 1992; Bertoft, Manelius, Myllarinen, & Schulman, 2000; Lauro, Forssell, Suortti, Hulleman, & Poutanen, 1999; Li, Vasanthan, Hoover, & Rossnagel, 2004; MacGregor & Ballance, 1980; MacGregor & Morgan, 1986; Maeda, Kiribuchi, & Nakamura, 1978), relatively little is known about the molecular structure and physicochemical properties of polymers remaining in the enzyme-modified granules. Also, to our knowledge, the acid hydrolysis of barley starches in the presence of various alcohols has not yet been conducted. In the present work, granular hulless barley starches with various amylose contents were modified by α-amylase and acid/alcohol hydrolyses. The objectives of this work were to determine the effect of amylose content on the degree of enzyme and acid/alcohol hydrolysis and to determine the differences in the molecular characteristics of the starch material remaining in the granules after partial α-amylolysis compared to that after acid/alcohol hydrolysis.

2. Experimental

2.1. Materials

Three types of starches: normal, high amylose, and zero amylose, were isolated from three hulless barley genotypes: Falcon, CDC 92-55-06-48, and CDC Alamo, respectively, according to a previously reported procedure (You & Izydorczyk, 2002). The amylose content in isolated barley starches was determined by potentiometric titration (Schoch, 1967). Barley samples were de-fatted by extraction

in a Soxhlet apparatus with 3:1 (v/v) *n*-propanol: water for 16 h prior to the amylose determination. The amylose content in normal, high amylose, and zero amylose starch was 23.7%, 41.9%, and 0%, respectively, whereas the lipid content was 0.68%, 0.80% and 0.34%, respectively. The purity of the starch preparations was greater than 98.5% as revealed by the starch content of the samples (AACC method 76.13).

2.2. Preparation of partially hydrolyzed samples

α-Amylase (*Bacillus licheniformis*, Megazyme, Bray, Ireland) was added to the starch suspensions (0.5 g starch/10 mL of 0.1 M ammonium acetate, pH 6.0) to provide enzyme concentrations of 50 or 500 U per gram of starch. The suspensions were gently shaken (Labquake shaker, Labindustries, Berkeley, CA, USA) at 20 °C. After 24 and 48 h, the suspensions were centrifuged (6725*g*, 10 min) and the residual starch granules were re-suspended with a buffer (0.2 M Na_2HPO_4–0.1 M citric acid, pH 2.5) for 1 h to inactivate α-amylase. Subsequently, the suspensions were centrifuged (6725*g*, 10 min), re-suspended in a buffer (0.2 M Na_2HPO_4–0.1 M citric acid, pH 6.5), centrifuged (6725*g*, 10 min), washed with water and acetone, and then dried at room temperature. The amount of solubilized carbohydrates during α-amylolysis was determined from the yield of the residual granules as well as determined by further digestion of the supernatant with thermostable α-amylase (*Bacillus licheniformis*, #FAA, Ankom Tech. Corp., Fairport, NY, USA) and amyloglucosidase (*Aspergillus niger*, Boehringer–Mannheim, Laval, Quebec, Canada). The resulting glucose contents in the supernatant were measured with a Gluco-quant assay kit (Boehringer–Mannheim, Laval, Quebec, Canada), according to the procedure of Salomonsson, Theander, and Westerlund (1984).

The acid hydrolyzed samples were prepared by suspending starch granules (6 g) in methanol, ethanol or 1-butanol (24 mL), and adding concentrated HCl to achieve 0.36% and 2% acid concentration. Samples were gently stirred at 20 °C for 10 and 96 h. The reaction was stopped by adding $NaHCO_3$, and the suspensions were washed repeatably with 70% ethanol and filtered (0.45 μm HVLP membrane). The acid/alcohol-treated starches were washed with acetone and dried overnight at room temperature. The yield was calculated as a ratio of weight of starch after treatment/weight of starch before treatment ×100%.

2.3. M_w analysis of starch polymers

The native and partially hydrolyzed starches were solubilized in 90% dimethyl sulfoxide (DMSO), precipitated with ethanol, and then dried (Jane & Chen, 1992). The native and α-amylase treated samples were then re-dissolved in 1 N NaOH and subsequently neutralized (1 N HCl). The solutions were autoclaved (20 min, 121 °C), and filtered (3.0 μm cellulose acetate membrane). The acid/alcohol-modified starches (3 mg) were dissolved in boiling water (10 mL) for 10 min. The weight average molecular weight (M_w) of native and partially hydrolyzed starches was determined using high performance size exclusion chromatography (HPSEC) [pump (Waters 510), injection valve (Model 7010, Rheodyne), a guard column (TSK PWH, Tosoh Corp.), a TSK G5000 PW analytical column (7.8 × 600 mm, TSK PW, Tosoh Corp.)] with multiangle light scattering (MALS) (Dawn DSP, Wyatt Technology) and RI (Waters 410) detectors. The columns were maintained at room temperature. The flow rate of mobile phase (0.15 M $NaNO_3$ containing 0.02% NaN_3, filtered through 0.2 and 0.1 μm cellulose acetate membranes) was 0.4 ml/min. Calculations of M_w and R_g were performed using the Astra 4.72 software (Wyatt Technology). Pullulan standards with known M_w values (P-50, M_w: 47,300; P-400, M_w: 404,000; P-800, M_w: 788,000) were used to determine the proper experimental setup and calculations.

2.4. Debranching of native and partially hydrolyzed starches

The native and partially hydrolyzed starches (25 mg) were dissolved, as described above, and subsequently incubated with isoamylase (Hayashibara Biochemical Laboratories Inc., Okayama, Japan; 500 U/g, 0.1 M acetate buffer, pH 3.5) for 24 h at 40 °C. The debranched starches were analyzed by HPSEC-MALS-RI using TSK G5000 PW, G3000 PWXL, and G2500PWXL (7.8 × 300 mm) columns in series as well as by high performance anion exchange chromatography (HPAEC, Dionex Carbopac PAI column, Dionex Corp. Sunnyvale, CA, USA) with a pulsed amperometric detector (PAD, Dionex, PAD II, gold electrode, 10k nA output, Dionex Corp., Sunnyvale, CA, USA), as previously reported by MacGregor, Bazin, Macri, and Babb (1999).

2.5. Other physical and rheological measurements

Starch samples were coated in a Hummer VII (Anatech, Ltd., Springfield, VA, USA) sputter coater on a 45° holder with 40 nm of gold (coat with 20 nm, rotate the stubs 180°, and coat with another 20 nm of gold). Gold-coated starch samples were examined with a JEOL JSM-6400 scanning electron microscope (SEM) at 10 kV and photographed on Kodak TMAX 100 Black and White Professional film.

The differential scanning calorimetry (DSC) of native and enzyme-treated starches was carried out with a DSC 2920 (TA Instruments, New Castle, DE, USA). Starch samples (40% w/w) were heated from 25 to 130 °C (10 °C/min). The gelatinization onset (T_o), peak (T_p), and complete (T_c) temperatures were measured, and enthalpy (ΔH) was calculated using the TA analysis software (TA Instruments).

Rheological measurements were carried out using a stress-controlled rheometer (Rheometrics SR 500, Rheometrics Scientific Inc., Piscataway, NJ, USA) with a parallel plate geometry. Starch sample (40% w/w) was heated in a sealed container in boiling water for 20 min and cooled to 50 °C. The hot paste was placed between the plates and

covered with mineral oil to prevent any moisture loss. All measurements were conducted at a frequency of 0.5 Hz and a constant 0.5% strain by applying the autostress adjustment mode, while the starch paste was cooled from 50 to 5 °C (5 °C/min) and stored at 5 °C for 20 h. All analyses were conducted in triplicate. The coefficient of variation (CV) of G' and G'' values was less than 5% in all cases.

3. Results and discussion

3.1. Solubilization and morphology of enzyme- and acid/alcohol-treated barley starches

Differences in the amounts of starch materials recovered after α-amylolysis and acid–alcohol hydrolysis are shown in Table 1. Among the three different types of barley starches treated with two different concentrations of α-amylase (50 and 500 U/g) for 24 and 48 h, the greatest yield in each case was obtained for high amylose, followed by normal and zero amylose waxy starch. The restricted penetration of α-amylase into the high amylose granules, and, therefore, very low starch solubilization may be caused by a high granule density, limited swelling capacity, and/or higher amount of amylose–lipid complexes in these type of granules. Recently, Li, Vasanthan, Hoover, and Rossnagel (2003) proposed that waxy barley starches have wider intercrystalline amorphous growth rings and more open crystalline lamella than normal and high amylose granules.

In contrast to α-amylolysis, very little solubilization of barley starches occurred during acid/alcohol hydrolysis, which resulted in very high yields of treated starches (~91% to 99%). After 10 h of hydrolysis, the amount of solubilized carbohydrates from starch samples was below 2%, irrespective of the starch type and alcohol in which the hydrolysis was carried out. After 96 h of hydrolysis, only slightly larger amounts of carbohydrates were released from the zero amylose starches than from other starches. Very similar amounts of solubilized carbohydrates were obtained for hydrolysis conducted in methanol and ethanol. Overall, the lowest yield of treated starches was obtained when the acid hydrolysis was conducted in 1-butanol.

Table 1
Yield of granular starch remaining after partial enzymic and acid/alcohol hydrolysis

Treatment	Yield (% w/w)[a]		
	Normal	High amylose	Zero amylose waxy
α-Amylase 50 U/24 h	76.1 ± 6.1	86.6 ± 1.8	64.5 ± 1.4
α-Amylase 50 U/48 h	75.0 ± 4.5	87.5 ± 6.6	58.0 ± 2.9
α-Amylase 500 U/24 h	70.6 ± 3.2	82.1 ± 2.7	55.9 ± 2.0
α-Amylase 500 U/48 h	61.1 ± 2.2	79.9 ± 3.9	44.2 ± 0.9
2% HCl/MeOH/10 h	99.8 ± 0.2	99.7 ± 0.2	99.6 ± 0.3
2% HCl/EtOH/10 h	99.4 ± 0.6	99.6 ± 0.2	99.6 ± 0.2
2% HCl/BuOH/10 h	98.6 ± 0.8	98.4 ± 0.5	98.1 ± 0.6
2% HCl/MeOH/96 h	98.4 ± 0.3	98.5 ± 0.5	97.1 ± 0.6
2% HCl/EtOH/96 h	98.3 ± 0.3	98.4 ± 0.5	97.9 ± 0.6
2% HCl/BuOH/96 h	91.5 ± 1.8	92.3 ± 2.2	92.9 ± 2.8

[a] $n = 3$.

α-Amylase treatments significantly affected the appearance of zero amylose starch granules, creating sponge-like structures with large pores. Relatively uniform erosion was observed at 48 h of hydrolysis, even at the lowest enzyme concentration (Fig. 1e). The hydrolysis of normal starch granules was somewhat less uniform with the majority of granules showing intact appearance and only a few having numerous pinholes (Fig. 1a). The high amylose starch granules showed almost no evidence of enzyme erosion (Fig. 1c). The interior examination of granules revealed that waxy and normal starch granules exhibited both radial and tangential hydrolysis patterns, and also that the endoerosion was confined only to certain areas of the granules as shown by the channels penetrating right through the granules (Fig. 1b and f). The hydrolysis pattern in high amylose granules appeared to be quite different (Fig. 1d); however, relatively few high amylose granules were hydrolyzed, compared with normal or waxy starch. In contrast to α-amylase-hydrolyzed granules, the granular surface of starches after acid/alcohol hydrolysis macroscopically remained unchanged (Fig. 2). Similar observations have been made for potato and waxy and normal corn starches (Chang et al., 2006).

3.2. Molecular weight of partially hydrolyzed starches

The molecular weight (M_w) distributions of starch polymers remaining in the granules after partial enzymic and acid/alcohol hydrolysis of barley starches are shown in Figs. 3 and 4, respectively. For intact starches, the first fraction (F1) corresponded to the high molecular weight amylopectin, whereas the second fraction (F2) corresponded to amylose and/or the low molecular weight amylopectin fraction. For all three types of barley starches, some changes in the M_w distribution were observed after α-amylolysis; however, both starch polymeric fractions retained their macromolecular form (Fig. 3). In contrast, a substantial depolymerization, and consequently drastic changes in the M_w distributions occurred after acid/alcohol hydrolysis (Fig. 4). Tables 2–4 summarize the relative content and the average molecular weight of each starch fraction before and after the hydrolysis treatments.

For the α-amylase-treated starches, the greatest changes in the relative content and molecular weight of fractions F1 and F2 occurred for the zero amylose starch (Table 4). After ~56% solubilization (500 U/g and 48 h hydrolysis) about a threefold decrease in the M_w of F1 and a fivefold decrease of F2 were observed. The changes in the M_w were accompanied by a decrease in the content of F1 from 79.3% to 58.7% and an increase of F2 from 20.8% to 41.4% (Table 4). The hydrolysis of zero amylose barley starch with 50 U/g for 48 h and with 500 U/g for 24 h resulted in similar contents of F1 and F2, but slightly lower molecular weights of both fractions after hydrolysis with a higher enzyme content.

The content and M_w of F1 in normal starch also decreased after α-amylolysis; however, the changes were

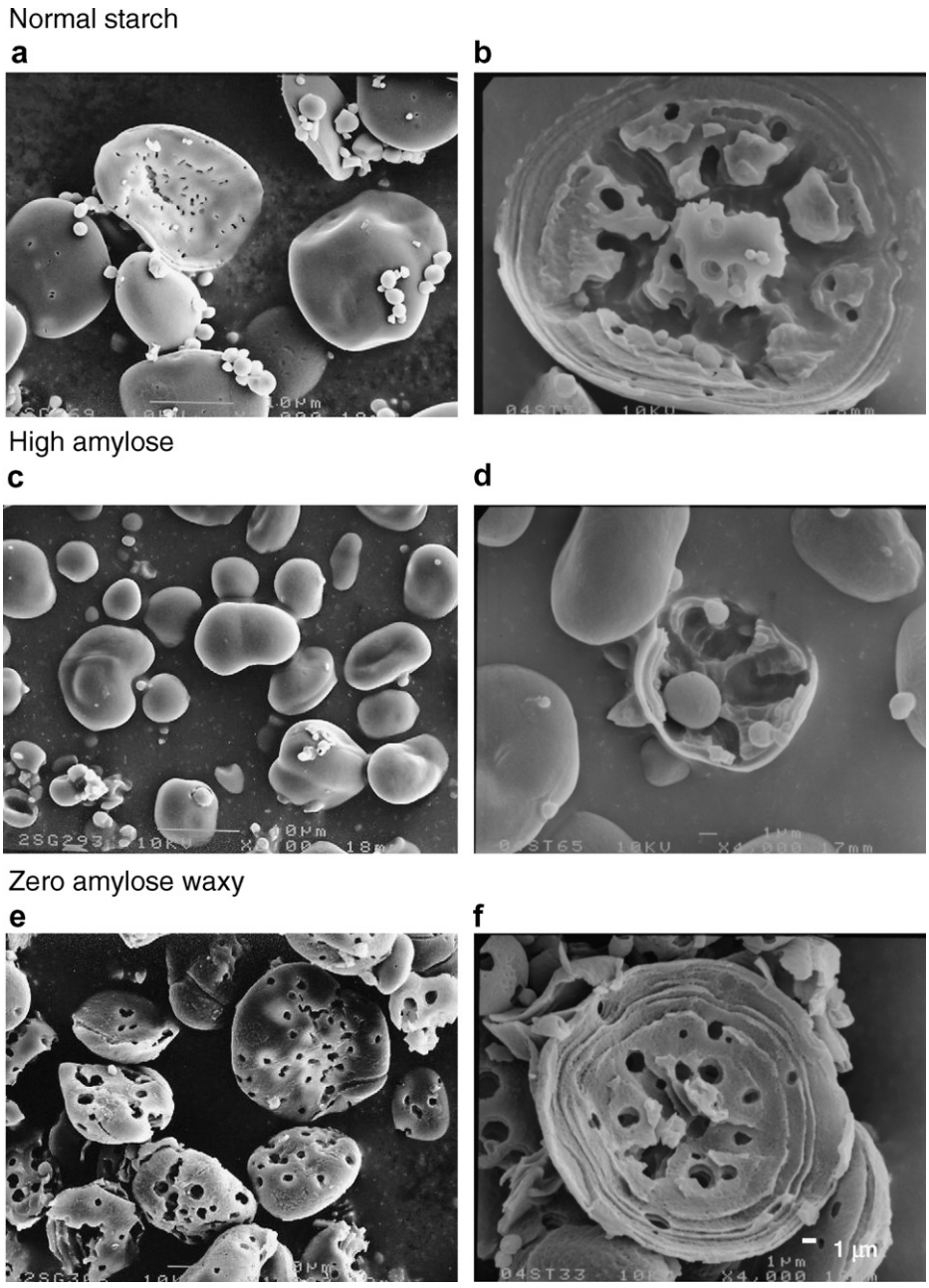

Fig. 1. Scanning electron photomicrographs of enzyme treated normal (a and b), high amylose (c and d), zero amylose waxy (e and f) barley starches (50 U α-amylase/g of starch, 48 h).

less drastic than those for the zero amylose starch (Table 2). The higher content of F2 in the partially hydrolyzed normal starch indicates that some partially hydrolyzed amylopectin eluted in this region. Among the three different starches, the high amylose starch was the least affected by α-amylolysis; only very small changes in the content and M_w of F1 and F2 were observed (Table 3). Contrary to our results, Lauro et al. (1999) reported that when half of the barley large granules were solubilized by α-amylase, the high molecular weight amylopectin peak disappeared from the HPSEC profile. On the other hand, Colonna, Buleon, and Lemarie (1988) reported that even after 91% solubilization, the molecular size distribution of wheat starch polymers was similar to that of the original starch. The authors suggested, however, that unequal susceptibility of wheat starch granules to α-amylolysis and the granule-by-granule hydrolysis mechanism may partially explain the unchanged molecular size distribution of starch polymers. Lauro et al. (1999) reported more uniform α-amylolysis of large barley starch granules, although they also observed some intact granules remaining after extensive hydrolysis. In our studies, the normal starch granules exhibited somewhat unequal susceptibility to hydrolysis, but the zero amylose waxy granules were uniformly eroded.

For the acid/alcohol treated starches, the relative amounts of fractions F1 and F2 and their degree of depolymerization

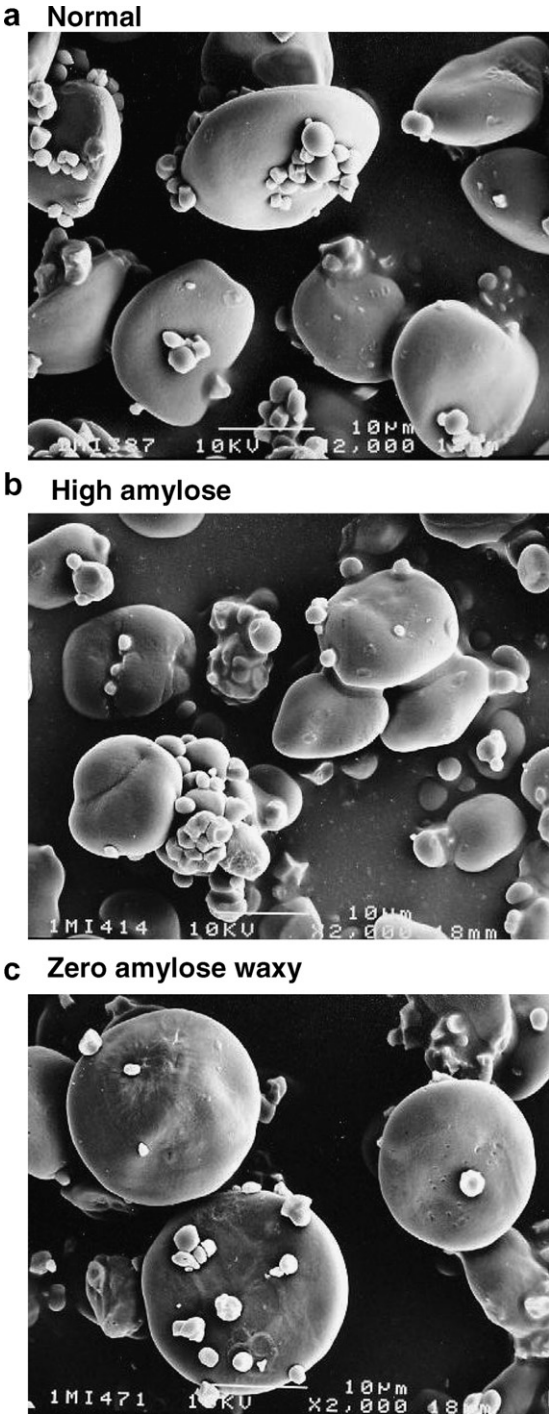

Fig. 2. Scanning electron photomicrographs of acid/alcohol treated (2% HCl/BuOH, 10 h) normal (a), high amylose (b), zero amylose waxy (c) barley starches.

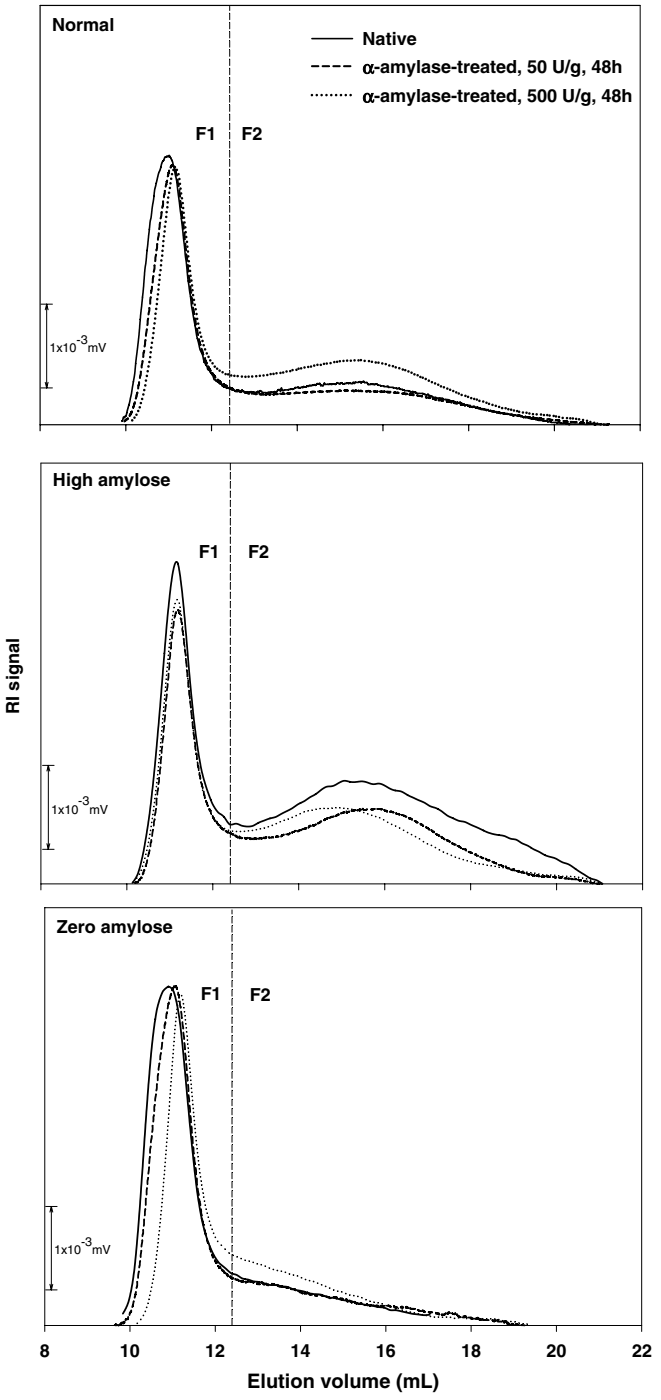

Fig. 3. HPSEC elution profiles of native and α-amylase-treated normal, high amylose, and zero amylose waxy barley starches.

depended on the hydrolysis time and the type of alcohol in which the hydrolysis was carried out. Generally, the greatest depolymerization occurred when the hydrolysis was conducted in 1-butanol. Acid hydrolysis in methanol and ethanol produced similar starch hydrolyzates, although small differences were noticeable. The changes in the molecular weight of starch polymers, observed in this study, were attributed to the combined effects of acid hydrolysis in the presence of various alcohols. However, no differences in the molecular weight distribution of all barley starches were observed in this study when starches were treated in alcohols (20 °C, 10 and 96 h) without acid (results not shown). The granular and molecular structure of starch may be affected by treatment of starches in alcohol at very high temperatures (Jane, Craig, Seib, & Hoseney, 1986). For example, corn starch granules heated under autogenic pressure (in closed vessels) from 230 to

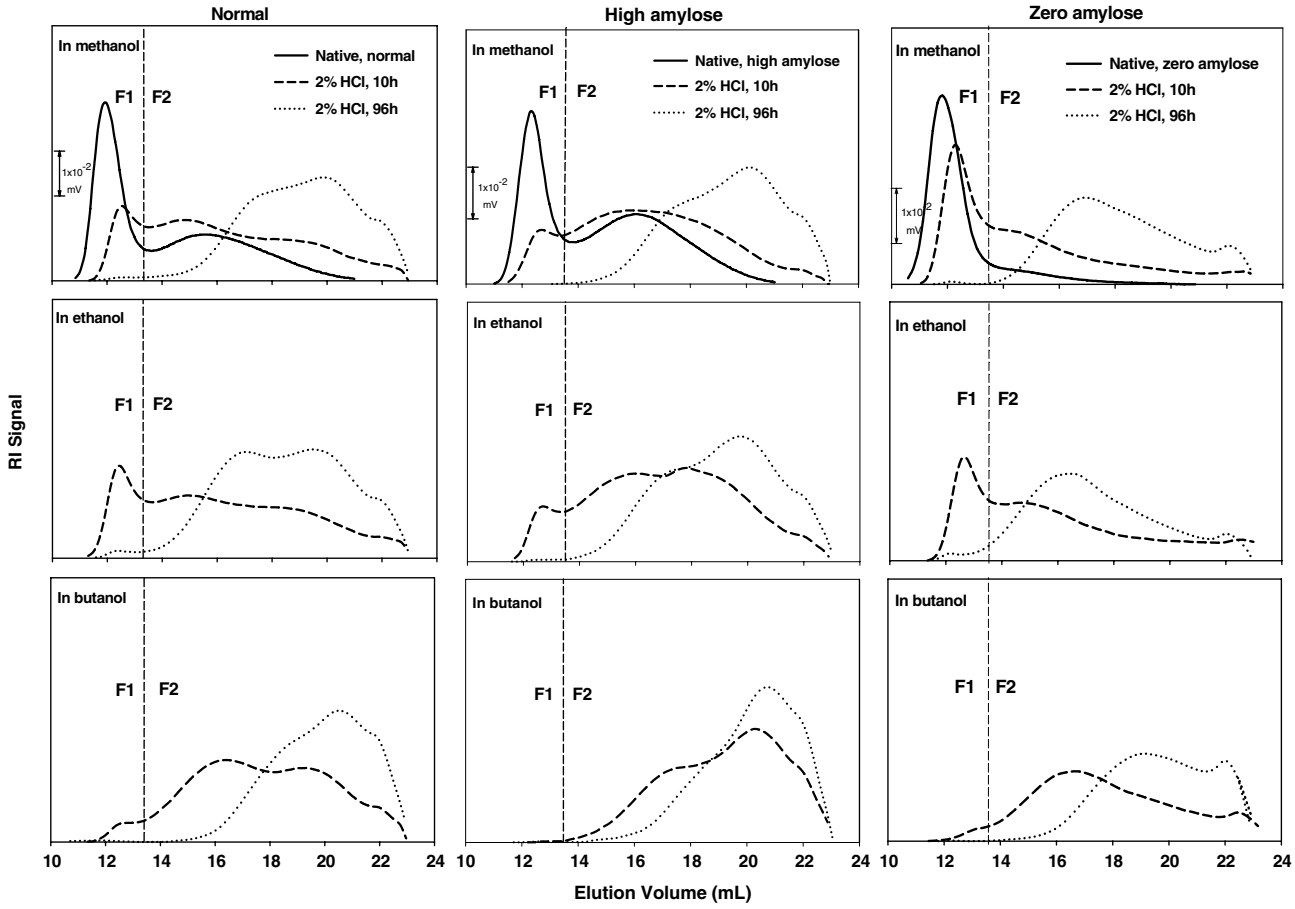

Fig. 4. HPSEC profiles of native and acid/alcohol-treated normal, high amylose, and zero amylose waxy barley starches.

Table 2
Relative content and weight average molecular weight of starch fractions in native and partially hydrolyzed normal barley starch

Starch/treatment	F1[a]			F2[a]			HPSEC recovery (%)
	%	$M_w \times 10^{-6}$	R_g (nm)	%	$M_w \times 10^{-6}$	R_g (nm)	
Normal starch							
Native	65.5 ± 4.2	114 ± 1.4	161 ± 0.7	34.6 ± 4.2	9.8 ± 0.4	124 ± 7.5	84.3 ± 0.9
α-Amylase 50 U/24 h	63.7 ± 3.5	87 ± 5.6	146 ± 4.2	36.3 ± 3.5	6.5 ± 0.3	112 ± 2.1	87.5 ± 3.5
α-Amylase 50 U/48 h	58.6 ± 4.2	78 ± 8.9	151 ± 13	41.4 ± 4.2	5.5 ± 0.6	112 ± 3.5	82.1 ± 0.4
α-Amylase 500 U/24 h	56.6 ± 2.4	83 ± 4.2	145 ± 2.8	43.4 ± 2.4	4.7 ± 0.3	106 ± 0.0	82.6 ± 1.6
α-Amylase 500 U/48 h	52.6 ± 7.7	82 ± 6.3	148 ± 22	47.5 ± 7.7	4.6 ± 2.8	104 ± 18	79.8 ± 8.8
2% HCl/MeOH/10 h	23.2 ± 1.3	43.2 ± 2.5	101 ± 3.2	76.8 ± 1.3	3.4 ± 0.4	51 ± 7.5	90.3 ± 3.5
2% HCl/EtOH/10 h	21.6 ± 2.4	37.3 ± 1.9	84 ± 4.1	78.4 ± 2.4	2.9 ± 0.4	52 ± 7.5	86.3 ± 2.0
2% HCl/BuOH/10 h	3.7 ± 0.2	nd	nd	96.3 ± 0.2	1.4 ± 0.4	42 ± 7.5	86.5 ± 2.5
2% HCl/MeOH/96 h	1.6 ± 0.3	nd	nd	98.4 ± 0.3	0.31 ± 0.0	35 ± 2.5	92.3 ± 3.0
2% HCl/EtOH/96 h	1.2 ± 0.1	nd	nd	98.8 ± 0.1	0.80 ± 0.1	44 ± 1.2	99.0 ± 0.5
2% HCl/BuOH/96 h	0	nd	nd	100 ± 0.0	0.15 ± 0.0	38 ± 1.3	93.8 ± 3.5

[a] The high- (F1) and low-molecular weight (F2) fractions as indicated in Fig. 3.

330 °F in ethanol/H_2O (3:1) underwent some chain cleavage and disruption of double helices (Eastman & Moore, 1984; Jane et al., 1986). Jane et al. (1986) postulated that heating starch granules with aqueous alcohol to a high enough temperature converts the double helical structure into single helices, and upon the eventual removal of alcohol renders the granules soluble in cold water. Lin et al. (2005) treated waxy maize and potato starches in methanol and 2-propanol at 65 °C for 1 h and observed some loss of the native order in granules (loss of 'Maltese cross') but no molecular degradation or changes in the pasting properties of the treated starches.

When the acid hydrolysis of barley starches was carried out for 10 h, all starches hydrolyzed in methanol exhibited a slightly higher average M_w of F1 and F2 than those hydrolyzed in ethanol (Tables 2–4). However, when the hydrolysis was carried out for 96 h, the average M_w of fraction F2 of starches hydrolyzed in ethanol was slightly higher than the average M_w of F2 of starches hydrolyzed in methanol (Fig. 4 and Tables 2–4).

Table 3
Relative content and weight average molecular weight of starch fractions in native and partially hydrolyzed high amylose barley starch

Starch/treatment	F1[a]			F2[a]			HPSEC recovery (%)
	%	$M_w \times 10^{-6}$	R_g(nm)	%	$M_w \times 10^{-6}$	R_g(nm)	
High amylose starch							
Native	44.5 ± 6.3	82 ± 0.7	121 ± 3.5	55.6 ± 6.3	3.8 ± 0.7	93 ± 2.8	79.5 ± 9.2
α-Amylase 50 U/24 h	43.9 ± 3.4	79 ± 6.2	121 ± 8.4	56.1 ± 3.4	3.6 ± 0.7	95 ± 4.2	79.2 ± 0.5
α-Amylase 50 U/48 h	43.0 ± 0.6	82 ± 7.2	120 ± 7.8	57.0 ± 0.6	3.4 ± 0.3	82 ± 0.7	80.3 ± 8.2
α-Amylase 500 U/24 h	43.2 ± 4.9	82 ± 5.9	119 ± 6.3	56.8 ± 4.9	3.5 ± 0.6	84 ± 2.8	84.5 ± 5.6
α-Amylase 500 U/48 h	43.3 ± 1.2	76 ± 9.3	115 ± 5.6	56.7 ± 1.2	3.1 ± 0.3	73 ± 4.2	84.3 ± 8.8
2% HCl/MeOH/10 h	12.9 ± 0.8	45.7 ± 5.1	95 ± 6.0	87.1 ± 0.8	3.5 ± 0.1	53 ± 2.5	85.6 ± 4.0
2% HCl/EtOH/10 h	9.4 ± 0.5	40.4 ± 8.2	81 ± 4.7	90.6 ± 0.5	2.6 ± 0.1	44 ± 1.2	89.8 ± 5.0
2% HCl/BuOH/10 h	0	nd	nd	100 ± 0.1	0.5 ± 0.0	34 ± 1.1	88.4 ± 2.0
2% HCl/MeOH/96 h	0	nd	nd	100 ± 0.0	0.35 ± 0.0	37 ± 1.0	82.4 ± 2.0
2% HCl/EtOH/96 h	0	nd	nd	100 ± 0.0	0.55 ± 0.1	38 ± 0.8	88.4 ± 2.5
2% HCl/BuOH/96 h	0	nd	nd	100 ± 0.0	0.14 ± 0.0	37 ± 0.3	83.1 ± 3.5

[a] The high- (F1) and low-molecular weight (F2) fractions as indicated in Fig. 3.

Table 4
Relative content and weight average molecular weight of starch fractions in native and partially hydrolyzed zero amylose waxy barley starch

Starch/treatment	F1[a]			F2[a]			HPSEC recovery (%)
	%	$M_w \times 10^{-6}$	R_g (nm)	%	$M_w \times 10^{-6}$	R_g (nm)	
Zero amylose waxy starch							
Native	79.3 ± 2.8	147 ± 8.5	186 ± 8.5	20.8 ± 2.8	31.0 ± 2.8	141 ± 15	79.7 ± 6.2
α-Amylase 50 U/24 h	75.0 ± 8.3	110 ± 2.8	165 ± 2.1	25.1 ± 8.3	21.8 ± 7.3	109 ± 8.4	77.0 ± 7.1
α-Amylase 50 U/48 h	75.7 ± 4.9	96 ± 9.1	159 ± 9.2	24.3 ± 4.9	18.1 ± 1.6	105 ± 13	84.6 ± 5.6
α-Amylase 500 U/24 h	74.2 ± 6.9	75 ± 1.4	140 ± 8.4	25.8 ± 6.9	15.8 ± 4.5	84 ± 0.7	82.5 ± 8.5
α-Amylase 500 U/48 h	58.7 ± 5.4	50 ± 0.3	115 ± 0.0	41.4 ± 5.4	5.9 ± 1.7	69 ± 6.4	84.0 ± 11.4
2% HCl/MeOH/10 h	45.9 ± 3.2	59.7 ± 4.2	105 ± 3.8	54.1 ± 3.2	6.4 ± 0.5	71 ± 1.2	100 ± 1.0
2% HCl/EtOH/10 h	32.8 ± 2.6	46.9 ± 4.9	96 ± 5.1	67.2 ± 2.6	3.6 ± 0.2	55 ± 0.5	99.3 ± 2.0
2% HCl/BuOH/10 h	3.6 ± 0.2	nd	nd	96.4 ± 0.2	1.5 ± 0.1	38 ± 0.9	100 ± 1.0
2% HCl/MeOH/96h	1.5 ± 0.1	nd	nd	98.5 ± 0.1	0.90 ± 0.1	54 ± 0.1	100 ± 1.0
2% HCl/EtOH/96 h	2.0 ± 0.1	nd	nd	98.0 ± 0.1	1.90 ± 0.1	53 ± 0.1	100 ± 1.0
2% HCl/BuOH/96 h	0	nd	nd	100 ± 0.0	0.34 ± 0.0	39 ± 0.3	100 ± 1.0

[a] The high- (F1) and low-molecular weight (F2) fractions as indicated in Fig. 3.

When the hydrolysis was carried out in methanol or ethanol for 10 h, the partially hydrolyzed starch granules contained a considerable amount of fraction F1, but very little of this fraction remained after 96 h hydrolysis (Tables 2–4, Fig. 4). For the normal starch granules treated for 10 h with 2% HCl in methanol and ethanol, the content of F1 changed from 65.5% to 23.2% and to 21.6%, respectively, which represented ~64% and 67% loss of fraction F1. The highest relative degradation of F1 occurred for the high amylose starch; the content of F1 changed from 44.5% to 12.9% and to 9.4% after 10 h hydrolysis in methanol and ethanol, respectively; these changes represented ~71% and 79% loss of fraction F1. The lowest losses of F1 occurred for the zero amylose starch; the content of F1 changed from 79.3 to 45.9 and to 32.8% after 10 h hydrolysis in methanol and ethanol, respectively, which represented ~42% and 59% loss of fraction F1. The average molecular weight of species eluting in fraction F1 also decreased after acid/alcohols treatments. About 2.5 to 3-fold decrease of M_w of the F1 fraction occurred for normal and zero amylose starches after 10 h hydrolysis in methanol and ethanol and about twofold decrease for the high amylose starch. The degradation of F1 was concomitant with the increasing amount of fraction F2. The species eluting in fraction F2 contained either a mixture of partially depolymerized amylopectin and amylose chains (normal and high amylose starches) or only depolymerized amylopectin chains (zero amylose waxy starch). For all partially hydrolyzed samples, the species eluting in fraction F2 had considerably lower M_w than those originating from the intact starches, but relatively high values of R_g. This may indicate more linear and extended conformation of the partially hydrolyzed chains compared to the relatively compact and branched structure of the intact starch polymers eluting in the same regions.

When the hydrolysis was carried out in 1-butanol, the majority of fraction F1 diminished even if the reaction was conducted for 10 h. These results indicate that in the presence of 1-butanol, acid penetration throughout the granule occurred very quickly and affected the whole population of starch polymers. Robyt and co-workers (Ma & Robyt, 1987; Robyt et al., 1996) suggested that higher alcohols might increase the actual concentration of acid inside the granules and/or assist in dissolution of the amylose double helices or amylose–amylopectin complexes and thus increase the overall hydrolysis rate and extent. This suggestion can be supported also by the fact that 1-butanol is less

miscible with water than methanol and ethanol. Our results are in good agreement with those of Robyt and co-workers (Fox & Robyt, 1992; Ma & Robyt, 1987; Robyt et al., 1996) who showed that by selecting different alcohols, concentration of acid and/or starch substrate, and temperature of hydrolysis reactions, partially hydrolyzed starches with a wide range of DP can be produced.

The average M_w and the M_w distribution profiles of starch populations (F1 and F2) obtained after acid/alcohol treatments did not indicate that the high amylose starch was less affected by hydrolysis than normal or waxy starches. This is contrary to previous reports (Li et al., 2001b; Vasanthan & Bhatty, 1996) which indicated that the extent of acid hydrolysis followed the order: waxy > normal > high amylose starch. However, the susceptibility of starches to hydrolysis has often been inferred from the amount of material solubilized and leached out of granules and not from the properties of the material remaining in the granules after hydrolysis. In addition, the hydrolysis conducted in the previous studies (Li et al., 2001b; Vasanthan & Bhatty, 1996) was performed under much harsher conditions (2.2 N HCl, 35 °C, 18 days). In our study, the waxy samples also produced a slightly higher amount of solubilized carbohydrates, but a close examination of the starch polymers inside the granules after hydrolysis did not indicate significant differences between waxy and high amylose starches in their susceptibility to hydrolysis under mild conditions as conducted in this study.

Despite the intact appearance of granules after partial acid/alcohol hydrolysis compared to the corroded granules after α-amylolysis (especially with the zero amylose starch), a greater modification of starch polymers occurred inside the granules due to acid/alcohol than enzyme hydrolysis. As previously suggested, acid seems to freely penetrate the entire granule and to hydrolyze the more susceptible glycosidic linkages located in the amorphous region, causing substantial decrease in M_w of starch polymers (Biliaderis, Grant, & Vose, 1981; Kainuma & French, 1971; Robin, Mercier, Charbonniere, & Guilbot, 1974). α-Amylase, on the other hand, due to its relatively large size (diameter of 6 nm), cannot easily diffuse into the granule and its action is confined to localized regions only, where both amorphous and crystalline regions are hydrolyzed concomitantly (Colonna et al., 1988; Leach & Schoch, 1961). Our results also indicated that the presence and the amount of amylose in starch granules affected the extent of degradation of barley starches and their susceptibility to enzymic but not to acid/alcohol hydrolysis.

3.3. Debranching of partially hydrolyzed starches

The HPSEC elution profiles of native, enzyme- and acid/alcohol-hydrolyzed normal and high amylose starches after debranching with isoamylase are shown in Fig. 5. Only small changes in the M_w of amylose were observed after α-amylolysis (Table 5). These results confirmed that amylose in barley starches was not preferentially hydrolyzed during α-amylolysis. Similar observations were made for wheat, corn, and sorghum starches (Colonna et al., 1988; Leach & Schoch, 1961). In contrast, a substantial degradation of amylose chains occurred after acid/alcohol treatments (Fig. 5, Table 5). The degradation of amylose depended on

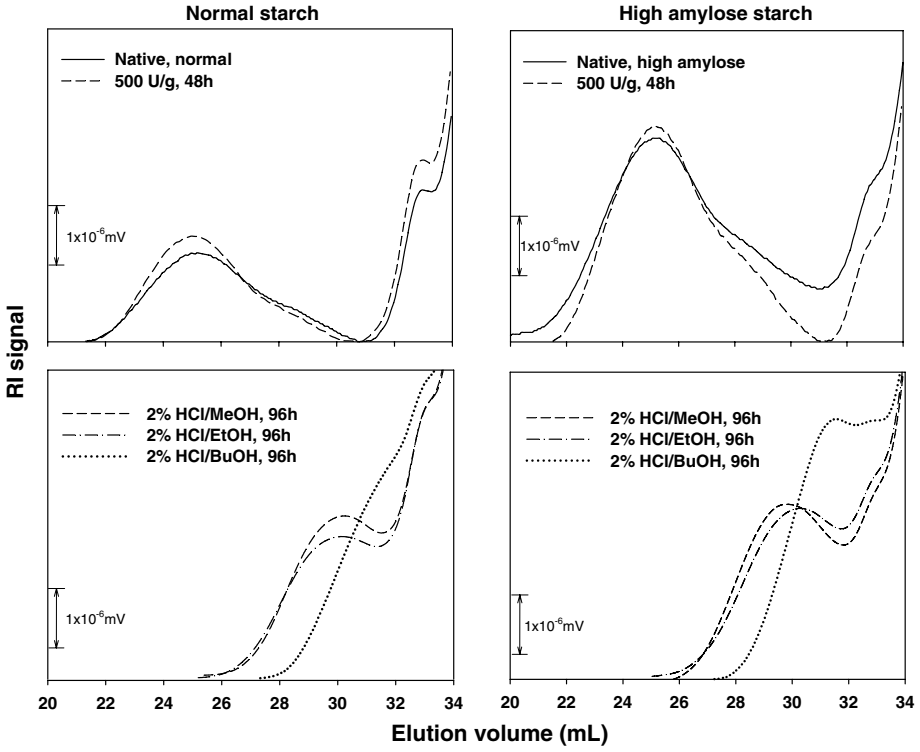

Fig. 5. HPSEC profiles of debranched amylose chains of native and enzyme and acid/alcohol-treated normal and high amylose barley starches.

Table 5
Weight average molecular weight of amylose fraction in native and partially hydrolyzed barley starches after debranching

Starch/treatment	Average mol wt $M_w \times 10^{-3}$ (V_e: 21–32 ml)	Mol wt at peak $M_w \times 10^{-3}$
Normal starch		
Native	370 ± 1.5	360 ± 1.2
α-Amylase 500 U/48 h	354 ± 2.9	369 ± 0.5
2% HCl/MeOH/96 h	50.4 ± 0.2	35.6 ± 0.1
2% HCl/EtOH/96 h	50.3 ± 0.3	36.7 ± 0.3
2% HCl/BuOH/96 h	32.5 ± 0.1	26.9 ± 0.1
High amylose starch		
Native	324 ± 0.7	363 ± 0.2
α-Amylase 500 U/48 h	305 ± 10	319 ± 2.5
2% HCl/MeOH/96 h	53.6 ± 0.3	42.5 ± 0.2
2% HCl/EtOH/96 h	74.8 ± 0.2	56.6 ± 1.1
2% HCl/BuOH/96 h	28.2 ± 0.2	20.2 ± 0.1

the alcohol medium in which the hydrolysis was conducted. The most severe depolymerization occurred when the acid hydrolysis was conducted in 1-butanol. The substantial decrease in the M_w of amylose chains during acid/alcohol hydrolysis indicates a pronounced susceptibility of amylose to hydrolysis. Ma and Robyt (1987) reported a significant decrease in DP of amylose polymers in potato starches hydrolyzed (0.36% HCl, 1 h, 65 °C) in methanol and ethanol and a complete depolymerization of amylose when the hydrolysis was conducted in 2-propanol or 1-butanol. Based on these observations the authors questioned the presence of amylose chains in the crystalline regions of starch granules.

The length and distribution of the debranched linear chains in native, enzyme- and acid/alcohol-hydrolyzed samples were also determined by the HPAEC-PAD (results not shown). After the enzymic hydrolysis, the amount of chains with DP $\geqslant$35 decreased, whereas the amount of chains with DP <34 slightly increased compared to the native starches. However, the differences were very small, and it appears that the primary molecular structure of linear chains in amylopectin, after partial α-amylolysis, was not significantly affected. Also, very small changes in the distribution of linear chains after acid–alcohol hydrolysis were observed. In general, the amount of longer chains slightly decreased, whereas the amount of short chains with DP $\leqslant$15 slightly increased after hydrolysis. The small increases in the amount of shorter chains (DP ~14) could have resulted from the scission of longer chains, either those connecting neighboring crystalline clusters or those originating from defective crystallites. Small increases in the amount of very short chains (DP <7) could indicate hydrolysis in the amorphous regions enriched in branch linkages.

These results are in good agreement with the current understanding of the mechanism of acid action inside the granule (Biliaderis et al., 1981; Jacobs et al., 1998; Kainuma & French, 1971; Li et al., 2001b; Robin et al., 1974; Shi & Seib, 1992; Wang & Wang, 2001). It is thought that acid hydrolysis initially occurs in the amorphous regions, located between crystalline regions and consisting of (1) branching points, (2) B chains connecting two or more clusters (Hizukuri, 1986), and (3) linear amylose chains (Robin et al., 1974). The densely packed crystalline regions consisting of short B and A chains (French, 1984; Robin et al., 1974) were not affected by the acid hydrolysis.

3.4. Thermal properties

The thermograms of the native and enzyme-treated normal and high amylose starches were generally similar, but the melting enthalpy values (ΔH) for amylopectin and amylose–lipid complexes in the partially hydrolyzed samples were higher than those in the intact starches (Table 6). No distinct differences in the peak melting temperatures (T_p) of amylopectin were recorded, but the completion temperatures (T_c) were slightly higher for the partially hydrolyzed samples. According to Biliaderis, Maurice, and Vose (1980), Cooke and Gidley (1992), the ΔH values correlate mainly with the amount of double helical domains (amylopectin) and single helical structures (amylose–lipid complexes) that unravel and melt during heating of aqueous starch dispersions. Our results may indicate, therefore, that α-amylase preferentially hydrolyzed the weakly organized regions, leaving the regions enriched in double-helical order intact. It is also possible that some re-ordering and re-structuring of starch polymers within the granule may have also occurred during α-amylolysis (e.g. retrogradation of partially hydrolyzed amylose chains). In addition to higher enthalpy values for amylopectin, the partially hydrolyzed normal and high amylose starches also exhibited higher ΔH values for amylose–lipid complexes.

For the zero amylose barley starches, which underwent much greater solubilization than normal and high amylose samples during α-amylolysis, a greater increase in the T_p and ΔH values was observed. These results could suggest that the overall crystalline order in zero amylose starch increased due to partial α-amylolysis. This could be either due to a more extensive degradation of chains in the less dense amorphous regions of zero amylose starch granules compared to high amylose or normal granules, or due to perfection of the crystalline regions facilitated by plasticization and increased chain mobility in regions affected by α-amylase. To date, there is no clear agreement as to the effects of enzymic hydrolysis on the thermal properties of starches. Lauro et al. (1999) observed large increases in T_p, and decreases in ΔH with increasing solubilization rate of large barley starch granules, whereas Colonna et al. (1988) reported no changes in the DSC profiles of wheat starches, even after 91% solubilization. Recently, Zhou, Hoover, and Liu (2004) reported a small decrease of ΔH values in partially hydrolyzed legume starches.

For the acid/alcohol-treated starches, the peak melting temperature (T_p) of all samples decreased the most after 10 h but started to slightly increase after longer hydrolysis time (96 h) (Table 6). Shi and Seib (1992) also reported that the T_p of lintnerized waxy rice starches decreased during the first two days of hydrolysis and only then started to

Table 6
Gelatinization temperatures and enthalpies of native and partially hydrolyzed barley starches

Starch/treatment	Amylopectin			Amylose	
	T_p (°C)	T_c–T_o (°C)	ΔH (J/g)	T_p (°C)	ΔH (J/g)
Normal starch					
Native	64.1 ± 0.6	28.3 ± 1.5	10.6 ± 0.9	101.5 ± 0.6	1.9 ± 0.3
α-Amylase 500 U/24 h	63.7 ± 0.4	28.2 ± 0.5	12.2 ± 0.1	102.9 ± 0.2	3.1 ± 0.0
α-Amylase 500 U/48 h	64.9 ± 0.1	29.3 ± 0.2	11.9 ± 0.5	103.8 ± 0.4	4.6 ± 0.0
2% HCl/MeOH/10 h	60.0 ± 0.4	34.7 ± 0.2	10.0 ± 0.3	102.3 ± 0.1	1.8 ± 0.1
2% HCl/EtOH/10 h	61.0 ± 0.0	31.4 ± 1.7	10.1 ± 0.1	100.2 ± 0.2	2.7 ± 0.2
2% HCl/BuOH/10 h	63.0 ± 0.3	30.9 ± 0.8	9.3 ± 0.6	100.7 ± 0.1	3.1 ± 0.1
2% HCl/MeOH/96 h	63.8 ± 0.0	37.8 ± 1.1	9.9 ± 0.2	100.7 ± 0.7	0.7 ± 0.4
2% HCl/EtOH/96 h	62.2 ± 1.1	37.2 ± 3.7	9.8 ± 0.7	101.5 ± 1.6	1.5 ± 0.1
2% HCl/BuOH/96 h	64.0 ± 1.0	32.5 ± 3.3	5.5 ± 0.5	93.5 ± 1.9	1.6 ± 0.2
High amylose starch					
Native	66.3 ± 0.6	31.1 ± 1.7	8.0 ± 0.9	101.2 ± 0.2	2.7 ± 0.5
α-Amylase 500 U/24 h	65.4 ± 0.4	33.9 ± 0.2	8.5 ± 0.2	102.9 ± 0.3	3.5 ± 0.1
α-Amylase 500 U/48 h	65.2 ± 0.0	32.2 ± 0.1	9.6 ± 0.1	103.5 ± 0.0	4.2 ± 0.1
2% HCl/MeOH/10 h	61.5 ± 1.0	36.2 ± 1.0	7.3 ± 0.5	101.3 ± 1.1	2.2 ± 0.1
2% HCl/EtOH/10 h	62.7 ± 0.0	36.4 ± 0.4	8.1 ± 0.1	101.8 ± 1.3	3.3 ± 0.1
2% HCl/BuOH/10 h	63.2 ± 0.4	32.4 ± 4.8	4.1 ± 0.6	98.2 ± 0.9	2.2 ± 0.3
2% HCl/MeOH/96 h	62.8 ± 0.2	35.1 ± 2.3	7.0 ± 1.0	101.3 ± 0.2	0.9 ± 0.6
2% HCl/EtOH/96 h	62.9 ± 0.0	33.8 ± 1.7	6.8 ± 0.2	101.2 ± 0.9	2.4 ± 0.1
2% HCl/BuOH/96 h	63.9 ± 0.1	34.5 ± 3.2	4.6 ± 0.1	100.4 ± 0.8	0.9 ± 0.1
Zero amylose waxy starch					
Native	66.4 ± 0.5	30.2 ± 0.6	14.8 ± 0.7	–	–
α-Amylase 500 U/24 h	67.9 ± 0.7	28.1 ± 2.1	16.8 ± 0.3	–	–
α-Amylase 500 U/48 h	73.9 ± 0.1	30.3 ± 1.4	20.2 ± 0.3	–	–
2% HCl/MeOH/10 h	62.9 ± 0.4	36.2 ± 2.4	14.4 ± 0.7	–	–
2% HCl/EtOH/10 h	63.2 ± 0.4	37.6 ± 2.3	14.6 ± 0.5	–	–
2% HCl/BuOH/10 h	62.8 ± 0.7	38.1 ± 2.8	14.4 ± 0.3	–	–
2% HCl/MeOH/96 h	62.1 ± 0.1	47.0 ± 3.5	15.8 ± 0.2	–	–
2% HCl/EtOH/96 h	63.2 ± 0.1	45.5 ± 4.0	15.5 ± 0.3	–	–
2% HCl/BuOH/96 h	58.1 ± 1.7	59.1 ± 0.2	13.6 ± 0.4	–	–

T_p, T_o, and T_c stand for the peak, onset, and completion temperature of gelatinization, respectively.

increase. The peak width (T_c–T_o) generally broadened due to the hydrolysis. The enthalpy values (ΔH) of normal and high amylose samples decreased somewhat upon hydrolysis. The ΔH of amylose–lipid complexes decreased, indicating extensive depolymerization of amylose chains as shown in Fig. 4 and Table 5. The ΔH of zero amylose waxy samples did not change substantially upon hydrolysis. In general, upon extensive lintnerization of starches, a shift of the endotherms to higher temperature and a peak broadening, but no changes in enthalpy of gelatinization are expected (Biliaderis et al., 1980; Jacobs et al., 1998; Shi & Seib, 1992). These changes are attributed to the selective removal of the amorphous region in a granule by acid. The small decreases in the ΔH values, observed for the partially hydrolyzed normal and high amylose samples, may indicate degradation of some amylose chains involved in double helical structures with either other amylose or amylopectin chains.

3.5. Rheological properties of modified barley starches

The viscoelastic properties of native, enzyme- and acid/alcohol-treated starches (40% w/w) during storage for 20 h at 5 °C are shown in Fig. 6. Upon cooling and storage, the native normal and high amylose starch solutions exhibited a greater development of the elastic (G') than viscous (G'') modulus, indicating the formation of strong three-dimensional networks. During storage, the tanδ values decreased from 0.19 to 0.13 for normal starch and from 0.14 to 0.09 for the high amylose sample. The tanδ and G' values clearly indicated that a high amylose starch forms stronger gels than a normal amylose starch. The native zero amylose starch showed substantially lower G' values during cooling and storage than normal and high amylose samples, indicating the formation of a paste rather than a gel. A very slow retrogradation of waxy barley starch indicates rather interesting rheological properties of this starch. Czuchajowska, Klamczynski, Paszczynska, and Baik (1998) also reported slow recrystallization for waxy barley during storage when probed with the DSC technique. These poor gelation and retrogradation properties of waxy barley starches may be associated with the relatively short length of the linear chains in amylopectin of these starches (You & Izydorczyk, 2002).

To determine the effects of enzymatic modification on the gelation properties of various barley starches, we chose only the least hydrolyzed samples (50 U α-amylase/g of starch, 24 h hydrolysis) due to their relatively high yield. The

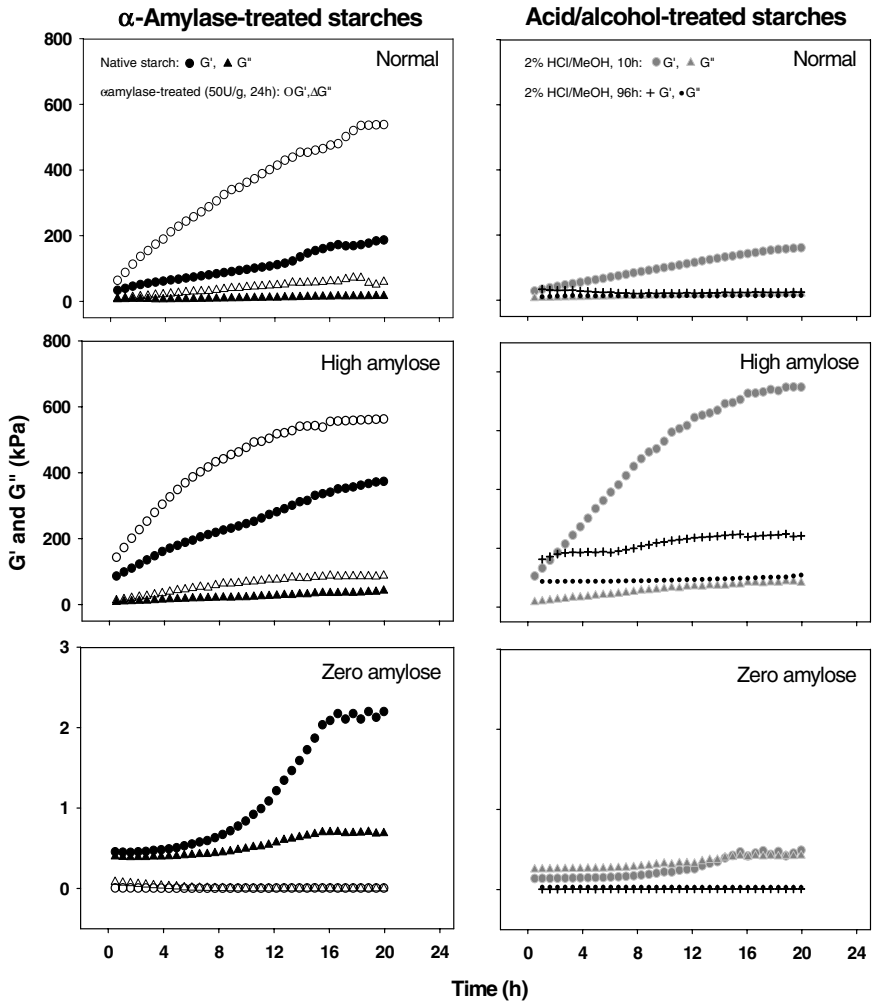

Fig. 6. Development of G' and G'' for native, α-amylase-and acid/alcohol-treated barley starches (40% w/w) upon cooling and storage from 50 to 5 °C (5 °C/min).

enzyme-treated normal and high amylose barley starches exhibited greater increases in G' values than intact starches during cooling and storage (Fig. 6). The 2% HCl/MeOH (10 h)-treated high amylose starch showed significantly higher values of G' compared to the native sample, whereas the treated normal starch exhibited the G' development similar to its native counterpart (Fig. 6). Both normal and high amylose starches, hydrolyzed for 96 h in 2% HCl/MeOH, exhibited a rapid rise in G' upon initial cooling (results not shown) but very little network development thereafter, resulting in very low values of G' and G'' compared to their native and 10 h-hydrolyzed counterparts. The substantial differences in the molecular weight of the 10 h- vs. 96 h-hydrolyzed samples (Tables 2 and 3, and Fig. 4) are responsible for the observed effects. The 2% HCl/MeOH (10 h)-treated high amylose starch exhibited stronger gelation properties than the enzyme-modified high amylose and normal starches. These results indicate that the optimum gelation potential of amylose-containing starches can be obtained by appropriate modification of their molecular weight.

It appears that the partial depolymerization of starch polymers increased the mobility and diffusion of chains, and therefore increased the rate and extent of cross-link formation, leading to formation of stronger gel networks. Clark, Gidley, Richardson, and Ross-Murphy (1989) showed that the molecular size of amylose chains significantly affected the kinetics of gelation and the rheological properties of amylose gel. For long amylose chains (DP 2550 and 2800), it was shown that the initial formation of relatively few cross-links retarded chain mobility and slowed down subsequent cross-linking and increases in G' values. In the present study, small decreases in the molecular weight of amylose and amylopectin in both barley starches facilitated faster and stronger gel formation in both cases.

In contrast to normal and high amylose barley starches, the partially degraded zero amylose barley starch did not show any potential to form elastic cross-linked networks during storage (Fig. 6). As shown in Table 4, α-amylolysis (50 U/g, 24 h) only slightly decreased the molecular weight of amylopectin chains compared with the more extensive degradation obtained via acid hydrolysis (2% HCl/MeOH, 10 h). It appears, however, that even these small changes significantly decrease the capacity of zero amylose barley starch to form

elastic cross-linked networks. Lauro, Ring, Bullt, and Poutanen (1997) also reported that stiffness of amylopectin gels decreased substantially with increasing level of amylopectin degradation. It appears that even a small fragmentation of amylopectin polymers during partial enzymic or acidic hydrolysis completely diminished their potential for gelation and retrogradation. These results indicate that partially modified waxy barley starches are resistant to retrogradation. This behaviour may be particularly useful in certain food applications and should be further investigated.

4. Conclusion

Significant differences in the solubilization, granule appearance, and degree of depolymerization of starch polymers were observed between enzyme and acid/alcohol hydrolyzed barley starches. The solubilization of granules and degradation of starch polymers during enzymic hydrolysis of barley starches were affected by the amount of amylose in starch granules. The amount of solubilized carbohydrates after α-amylolysis ranged from about 13% to 20% for high amylose, from 25% to 39% for normal, and from 36% to 56% for zero amylose waxy samples, depending on the hydrolysis time and the amount of enzyme. In contrast, very little solubilization (<9%) of barley granules occurred during acid/alcohol hydrolysis. Despite a very low degree of solubilization and almost intact granular morphology, much greater degradation of starch polymers occurred inside the granules after acid/alcohol than after enzymic hydrolysis. The analysis of the molecular weight distribution of partially degraded starch polymers inside the granules after acid/alcohol hydrolysis has not indicated significant differences between waxy and high amylose starches in their susceptibility to this type of hydrolysis. The results of this study indicate that the average M_w and the mode of distribution of various M_w species can be tailored to specific needs by choosing appropriate hydrolysis conditions. The rheology results revealed that the amylose content as well as the hydrolysis type and conditions significantly affect the gelation potential of barley starches. The rheological properties of aqueous starch dispersions can thus be controlled by selection of appropriate hydrolysis conditions, and exploited in specific application for new product development.

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