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Molecular Characteristics and Functional Properties of Barley Starches with Varying Amylose Content

Sang Guan You[1] and Sang Moo Kim[2†]

[1]Department of Food Science, University of Manitoba, Winnipeg, MB, R3T-2N2, Canada
[2]Faculty of Marine Bioscience & Technology, Kangnung National University, Gangwon 210-702, Korea

Abstract

Molecular structures and functional properties of starches isolated from normal, waxy, and zero amylose barleys were examined. Amylopectins from zero amylose starch had the largest molecular weight (M_w), whereas those from high amylose starch, the smallest. A good correlation between the M_w and the radius of gyration (R_g) was observed among amylopectins from various starches, indicating similar polymeric conformation in solution even with the differences in the M_w. The debranched amylopectin molecules from different types of barley starches exhibited similar profiles, implying that the packing geometry of double helices in the different types of barley starches may be similar. Zero amylose starch showed the highest peak viscosity (326 RVU) in RVA viscograms at lower pasting temperature (67.6°C), compared to normal and high amylose starches. Relationship between RVA peak viscosity and amylose content suggested that the presence of amylose inhibited the development of granular swelling of barley starches during cooking. A rapid retrogradation, traced by differential scanning calorimetry (DSC) and strain-controlled rheometry, occurred in the high amylose starch sample during storage, while zero amylose starch showed a very good resistance to retrogradation, indicating excellent storage stability.

Key words: barley starch, molecular weight, pasting property, retrogradation

INTRODUCTION

Barley is one of the most widely cultivated cereal crops that can provide valuable nutrients required by humans and domestic animals. Its high adaptability to various climates and growing conditions has led to worldwide production averaging 145.9 million tonnes per year over the last decade. Although relatively little effort has so far been made to increase and diversify barley utilization outside the malting and feed industry, recent studies which have elucidated the role of grains in prevention and treatment of many human diseases might revive interest in barley for food purposes (1,2).

Barley grain consists mainly of carbohydrates (78~83%), proteins (8~15%), and lipids (2~3%) (3). Starch is the major component of barley grain and the dominant constituent of barley flour, controlling the major characteristics of barley products. Starch is composed of two polysaccharides, amylose (AM) and amylopectin (AP), with AP constituting approximately 75% of the starch in normal barley (3). When the generic trait of waxy starch is present, starch contains 95~100% amylopectin. The other mutation increases the amylose content up to 40%. Several barley varieties have recently been developed with amylose content ranging from 0 to 40%. Variability in amylose/amylopectin ratio can significantly affect barley starch swelling and gelatinization as well as pasting and retrogradation properties.

Salomonsson and Sundberg (4) used gel permeation chromatography (GPC) with Sepharose CL-2B column for the analysis of amylose and amylopectin chain-length profiles of normal, waxy, and high amylose barley starches. The authors reported that the average chain lengths of high amylose debranched amylopectin chains were five glucose units longer than those of normal and waxy amylopectin chains. Tester et al. (5), on the other hand, found no variations in chain lengths of debranched amylopectin from normal, waxy, and high amylose barley starches.

Studying a variety of barley starches, Vasanthan and Bhatty (6) reported the highest gelatinization temperature for high amylose barley starch (65.7~68.4°C), followed by waxy (62.6~64.0°C), and normal starch (58.0~61.0 °C). However, very small differences in gelatinization temperature between high amylose (62.8~63.2°C) and normal barley starch (59.0~60.3°C) were reported by

†Corresponding author. E-mail: smkim@kangnung.ac.kr
Phone: +82-33-640-2343, Fax: +82-33-640-2340

Song and Jane (7). These authors also reported that amylopectins in all barley starches had relatively short-branched chain lengths and a low proportion of longer chains (DP 18~21), suggesting a defective crystalline structure responsible for the very low gelatinization temperature in barley starches. Waxy barley starch was reported to have a lower degree of retrogradation than waxy maize and waxy rice starches (8). It was postulated that starch retrogradation appeared to be directly proportional to the mole fraction of unit chains with DP 14~24 and inversely proportional to the mole fraction of DP 6~9.

Relationships between the chemical structures and the functional properties of these starches were of great interest. In this study, we analyzed the molecular weight (M_w) and the radius of gyration (R_g) of barley starch components by using high-performance size exclusion chromatography connected with light scattering and refractive index detectors (HPSEC-MALS-RI). The length and distribution of the debranched linear chains of amylopectins were determined by high performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD). The molecular structures of barley starches were compared with their cooking profiles and retrogradation properties.

MATERIALS AND METHODS

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 the previously reported procedure (9). Amylose content in de-fatted barley starches was determined by a potentiometric titration method (10). The chemicals used were all reagent grades.

Molecular characteristics of starch polymers

A average molecular weight (M_w) of starch polymers was determined using high-performance size exclusion chromatography (HPSEC, TSK G5000PW column, Tosoh BioSep) combined with multi-angle laser light scattering (MALS, Dawn DSP, Wyatt Technology) and refractive index (RI, Waters 410) detectors, as the method of You et al. (11) with a slight modification. The column was kept at room temperature. The flow rate of mobile phase (0.15 M $NaNO_3$ containing 0.02% NaN_3), which was filtered through 0.2 μm and then 0.1 μm of cellulose acetate membranes, was 0.4 mL/min. Calculations of M_w and R_g were performed by the Astra 4.72 software (Wyatt Technology) using the Berry extrapolation method. 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.

Granular starch was gelatinized and then purified with 90% DMSO and alcohol precipitation, as stipulated by the method of Jane and Chen (12). The purified starch (7 mg) was steeped in ethyl-alcohol (0.1 mL), redissolved in 1 N NaOH (1 mL), diluted with water (8 mL) and neutralized with 1 N HCl. The starch solution was autoclaved for 20 min (121°C), filtered through a 3.0 μm cellulose acetate membrane, and then injected into the HPSEC-MALLS-RI system.

The debranched chain length profiles of barley starch amylopectins were determined using 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) (13). The 3 mL of starch solution, dissolved as above, was incubated with isoamylase (*Pseudomonas amyloderamosa*, 59,000 U/mL, Hayashibara Biochemical Laboratories Inc., Okayama, Japan) at the enzyme concentration of 500 U/g by adding 1 mL of acetate buffer (0.1 M, pH 3.5) for 24 h at 40°C. After incubation, the starch-enzyme solution was neutralized with 1 N NaOH, heated in a boiling water bath for 5 min to inactivate isoamylase, filtered through a 0.45 μm membrane, and then injected into the HPAEC-PAD system.

Pasting properties of starches

Starch pasting properties were determined by using a Rapid Visco-Analyzer (RVA) (Newport Scientific, Sydney, Australia). Starch suspension (8%, w/w, 28 g total weight) was equilibrated at 50°C for 1 min and then heated at a rate of 6°C/min to 95°C and then maintained at that temperature for 5 min. The sample was then cooled to 25°C at a rate of 6°C/min. A constant rotating speed of the paddle (160 rpm) was used throughout the analysis.

Water holding capacity of starches

Water holding capacity of starches was measured according to the method of Sasaki and Matsuki (14) with a slight modification. The 0.1 g starch sample was weighed directly into a glass tube with coated screw caps and then 5 mL of distilled water was added. The slurry was heated at a desired temperature, varied between 50°C and 95°C for 30 min in the temperature regulated water bath. The mixture was cooled to room temperature and centrifuged (5000×g, 10 min). The supernatant was removed carefully and water holding capacity of starches was determined as weight of the sediment by an equation: Water holding capacity (%)=$\{(W_2-W_1)/W_1\}\times 100$, where W_2 is weight of the sediment (g); W_1 is

weight of db starch (g).

Retrogradation of starches

Retrogradation properties of starches were analyzed using a differential scanning calorimeter (DSC 2920, TA Instruments, New Castle, DE, USA). Starch samples (3.8 ~4.0 mg) were suspended in water (40% w/w) and hermetically sealed in DSC pans (TA Instruments, New Castle, DE, USA). The starch suspensions were heated from 25°C to 130°C with a heating rate of 10°C/min. The retrogradation study was performed following the same heating condition using the same gelatinized starch samples that had been stored at 6°C for 7 days.

The mechanical properties of starch gels (40%, w/w) were probed by the Bohlin VOR rheometer (Bohlin Reologi, Inc., Edison, NJ) operated with a parallel-plate geometry (30 mm) and a torque element of 93.2 g · cm. Measurements were carried out at 0.5 Hz, 8°C and strain less than 2%.

RESULTS AND DISCUSSION

Molecular characteristics of barley starches

Various types of barley starches gave different elution profiles from the size exclusion chromatography (SEC) column (Fig. 1). A good separation of the large and small molecular weight starch components was achieved by the HPSEC system, which enabled us to conduct the measurement of molecular weight (M_w) and radius of gyration (R_g) of the starch components. The first, high molecular weight peak in the HPSEC chromatograms, with concentration maxima at the elution volume (V_e) of approximately 12.0 mL, constitutes the majority of amylopectin polymers in each starch sample, whereas the second, low molecular weight peak, at V_e maximum about 16.0 mL, corresponds largely to amylose polymers. The relative proportion of the two peaks gives some indication of the amylose content in each type of starch. However, the determination of amylose content in the samples on the basis of HPSEC chromatographic data would likely lead to an overestimation of amylose content because some amylopectins also elute in the lower molecular weight region, traditionally assigned to amylose. Among the M_w of the high M_w polymers (amylopectins) reported in Table 1, amylopectins of zero amylose starch had the highest average molecular weight (3.05×10^8 g/mol), whereas those from high amylose starch the lowest (1.36×10^8 g/mol). It has been reported that in normal starch biosynthesis carbon flux at a form of ADP-Glc is partitioned between amylose and amylopectin polymers (15,16). In waxy mutants, granule-bound starch synthase I (GBSSI), primarily involved in amylose biosynthesis of starch, is missing and no amylose is synthesized (15,16). Therefore, it has been suggested that ADP-Glc might be exclusively incorporated into amylopectin molecules resulting in amylopectin molecules with larger M_w in waxy mutants (17). It was also postulated that space limitation in the normal starch granule due to the presence of amylose molecules (about 25% of by mass) results in smaller M_w of normal maize amylopectins (17). Regardless of the differences in the M_w of various types of barley amylopectins, a good correlation (r^2=0.96) was observed between the molecular weights of all amylopectins and their dimensions, as estimated from the radius of gyration (R_g), indicating that molecular conformation of amylopectins from various starches are similar.

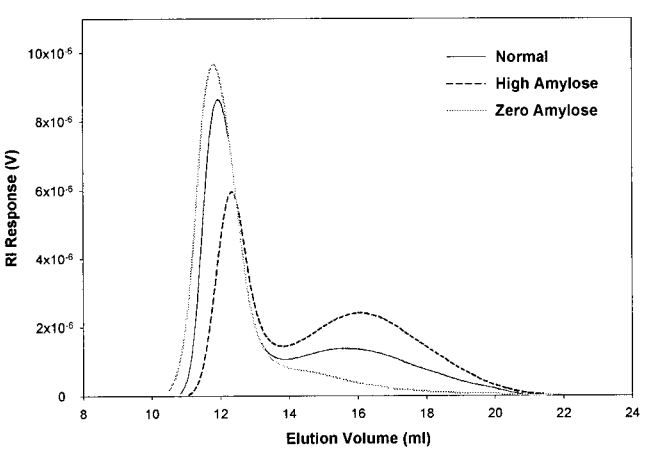

Fig. 1. High-performance size exclusion chromatograms for normal, high amylose, and zero amylose barley starches.

It is widely accepted that the methods of solubilization and separation used greatly affect the molecular weight of starch. Fishman et al. (18) reported M_w of waxy and normal maize amylopectins solubilized by microwave

Table 1. Molecular weights (M_w), radii of gyration (R_g), and amylose content of barley starches

Starch type	High M_w Fraction (amylopectin)		Low M_w Fraction (amylose)		Amylose content[1] (%)
	$M_w \times 10^{-6}$ (g/mol)	R_g (nm)	$M_w \times 10^{-6}$ (g/mol)	R_g (nm)	
Normal	226±9.9	223±4	5.67±0.90	107±4	23.7
High amylose	136±6.7	164±5	2.73±0.10	65±2	41.9
Zero amylose	305±0.7	266±1	40.7±13.0	148±17	0

[1]Determined by potentiometric titration with iodine.

heating in a high-pressure vessel to be 2.6×10^7 and 1.5×10^7, respectively. On the other hand, the M_w of jet-cooked waxy maize starch measured by Klavons et al. method (19) was found to be 4.12×10^8 in batch mode, but 2.24×10^8 by hydrodynamic columns. Hanselmann et al. (20) reported M_w of 3.60×10^8 for waxy maize starch obtained from sedimentation field flow fractionation. In order to determine properly the M_w of starches, complete solubilization of starch polymers, amylose and amylopectin, in an appropriate solvent must be ensured. The degradation of starch polymers must be also avoided. Degradation or incomplete disaggregation of starch polymers would lead to underestimation or overestimation of true M_w of starch polymers. The autoclave heating used in this study (121°C, 20 min) appeared to be an appropriate starch solubilization method, providing better HPSEC recovery and higher M_w for amylopectins than simple dissolution in hot water (data not shown).

The molecular weights of low M_w fractions (mostly amylose) from various barley starches are compiled in Table 1. Despite no distinctive difference in the elution volume (Fig. 1), the calculated M_w of amylose polymers in normal and high amylose starches were significantly different, with the average M_w of amylose in normal starch almost twice as high as that of high amylose starch (Table 1 and Fig. 2). The observed differences in the M_w of amylose polymers might arise from the fact that some of branched material (amylopectin polymers) eluted in this region traditionally assigned to amylose. This was apparent from the elution profiles of zero amylose starch, which showed a small but definite population of polymers eluting in the volumes assigned to amylose, despite the absence of amylose fraction in zero amylose starch sample. In addition, the M_w and R_g of the low M_w fraction in zero amylose starch remained very high (Table 1). Therefore, the accurate determination of molecular characteristics of amylose polymers in normal and high amylose starches may be problematic by the presence of amylopectins eluting in the same volumes.

The relative distribution of linear amylopectin chains after debranching by isoamylase was examined using the HPAEC-PAD system (Fig. 3). Amylopectins from various barley starches showed similar chain length distributions (Fig. 3). All the amylopectins had the same peak and shoulder at DP 12 and 20, respectively (Fig. 3). These results imply that the packing geometry of double helices in the different types of barley starches may be similar. Indeed, the X-ray diffractograms confirmed that the different types of barley starches exhibited A-type crystalline pattern (data not shown). In contrast to various types of maize starches (21), it is unique for barley starches that amylose polymers appeared not to sig-

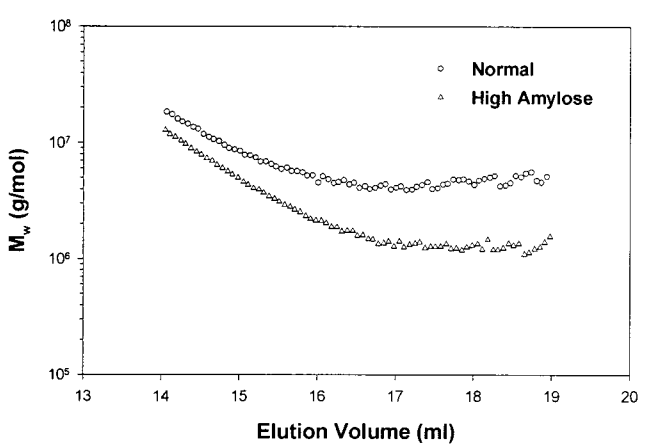

Fig. 2. Relative molecular weight (M_w) vs elution volume from HPSEC chromatograms for amylose polymers in normal and high amylose barley starches.

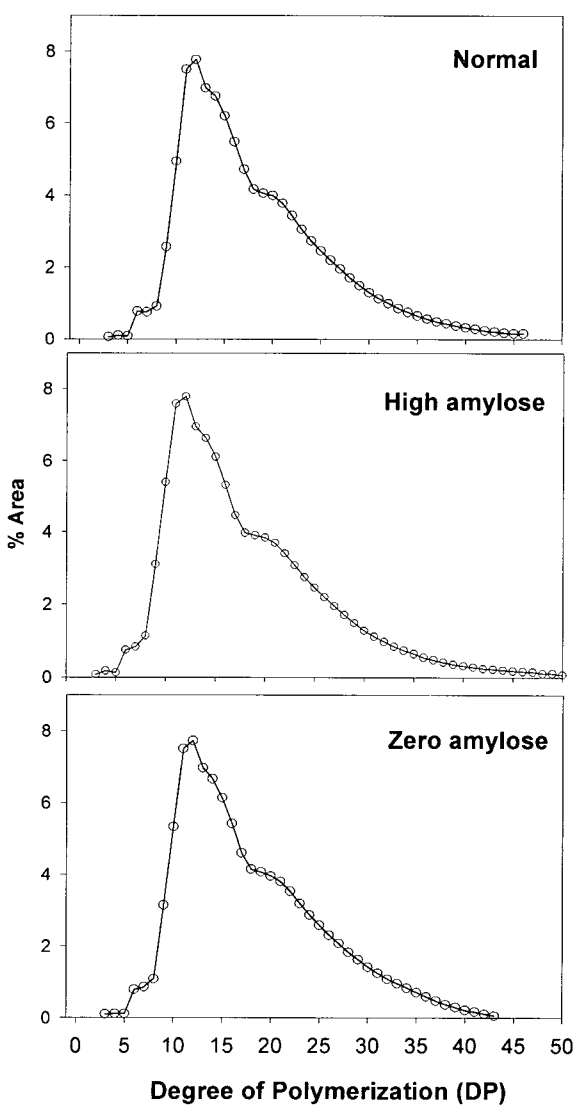

Fig. 3. HPAEC debranched chain length profiles of normal, high amylose, and zero amylose amylopectins.

nificantly influence the crystalline structure of starch granules. The longest detectable chains of amylopectins were observed for high amylose starch (up to DP 50), followed by normal and zero amylose starch (Fig. 3). Zero amylose starch had its longest detectable chain of DP 43. These results are not consistent with the results of Song and Jane (7), who reported that normal barley starch contained the longest linear chains with a DP value of 82. These discrepancies may be due to the differences in the origin of the starches as well as to differences in the methods employed for the detection of the debranched chains.

Pasting properties

RVA viscograms of barley starches with varying amylose content are shown in Fig. 4. Various types of barley starches showed distinctive differences in their pasting properties. Zero amylose starch had a low pasting temperature (67.6°C) and considerably higher peak viscosity (326.0 RVU) at shorter times than normal and high amylose starches. Normal barley starch had a pasting temperature at 90.7°C and substantially higher final viscosity (566.5 RVU). High amylose starch showed very low peak viscosity (28.3 RVU) and a high pasting temperature (94.9°C).

These results suggested that the granules from zero amylose starch were less resistant to swelling than those from normal and high amylose starches. High amylose starch, on the other hand, showed the smallest change in viscosity during heating and cooling cycle. Normal starch showed the highest consistency during cooling, indicating a greater retrogradation tendency than waxy and high amylose starches. The characteristic differences in pasting profiles among different types of barley starches can be due to the amylose content. Amylose polymers inside starch granules may serve as a connector among adjacent amylopectin molecules, preventing dissociation of amylopectin molecules and preserving the integrity of the granule (22). With no amylose, waxy starch granules could swell freely and develop a large peak viscosity in relatively short time. However, high amylose starch granules with 40% amylose content exhibited substantially reduced swelling capacity, showing the lowest peak viscosity.

Among the RVA parameters, a negative correlation ($r=-0.99$, $p<0.05$) was observed between the amylose content and the peak viscosity, which also suggests that the presence of amylose inhibit the development of viscosity in barley starches during cooking. Tester and Morrison (23) reported that amylopectin is primarily responsible for granule swelling. Similar results were also found in various types of barley starches (7,24).

Water holding capacity

Water holding capacity (WHC) of the starch granules was temperature-dependent as indicated in Fig. 5. WHC of starches increased with increase in temperature. At a low temperature, the water holding capacity of starches was not significantly different among the different types of barley starches. However, an increase in the heating temperature to more than 50°C led to the fact that WHC of zero amylose starch granules was the greatest, while that of high amylose starches the least. Zero amylose starch granules absorbed more water at higher temperature than normal and high amylose starches. These results correspond well to the pasting properties of barley starches, in which zero amylose starch showed the highest peak viscosity at a lower pasting temperature than normal and high amylose starches. The granular swelling could be directly related to the WHC of starch granule. The differences in WHC among various barley starches may be partly due to the presence of hydrocarbon chains

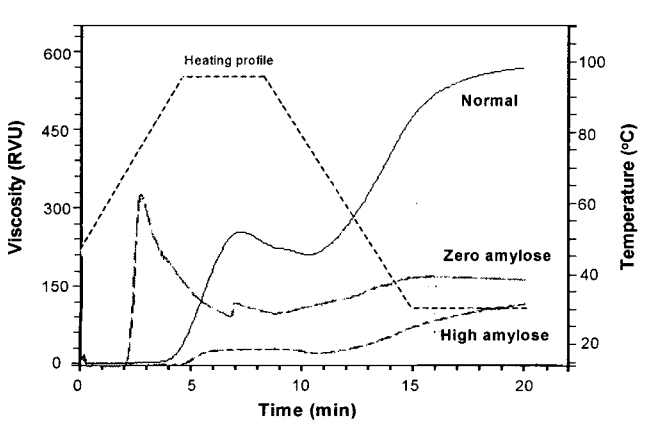

Fig. 4. RVA viscograms (8%, w/w) of normal, high amylose, and zero amylose barley starches.

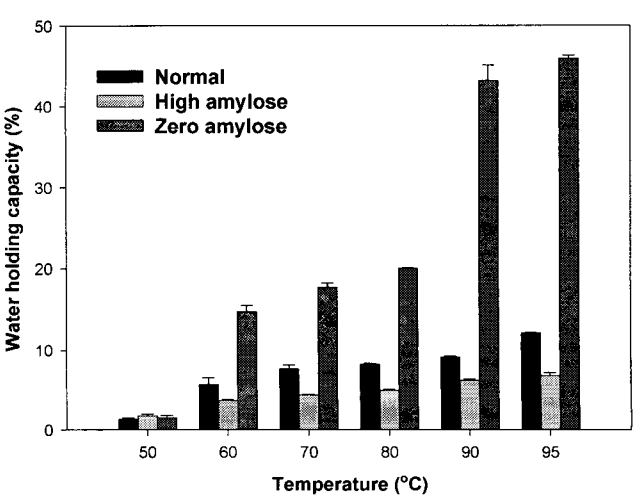

Fig. 5. Water holding capacity of normal, high amylose, and zero amylose barley starches.

of internal lipids, which suppress hydration of amorphous regions in starch granules (22).

Retrogradation of barley starches

When aqueous starch suspensions are heated above the gelatinization temperature, starch granules swell irreversibly, and lose their structural order (25). As the granules continue to expand with heating, amylose polymers leach out into the aqueous inter-granular phase, causing the substantial increase in viscosity (26). If the starch concentration is high enough, usually >6% w/w, the mixture of swollen granules and exuded amylose behaves as a viscoelastic paste. Upon cooling, the starch polymer mixture sets as a gel in which further thickening and rigidity development occur during storage as a result of chain aggregation or retrogradation (26). The retrogradation property of starches is one of the most important factors in the choice of the final application of starches in food industry.

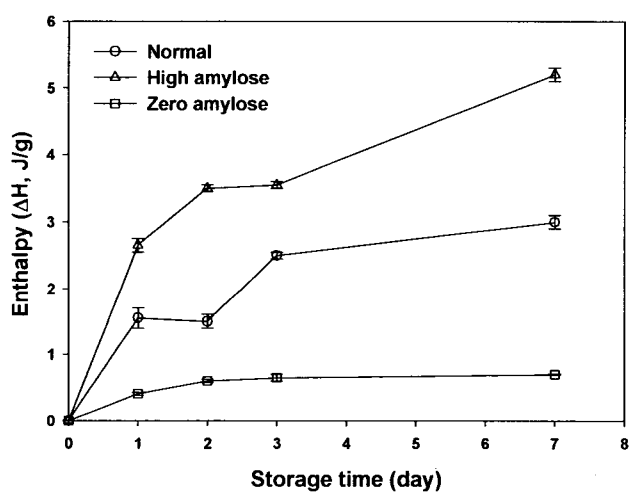

Fig. 6. Retrogradation kinetics of the gelatinized barley starches (40%, w/w) stored at 6°C.

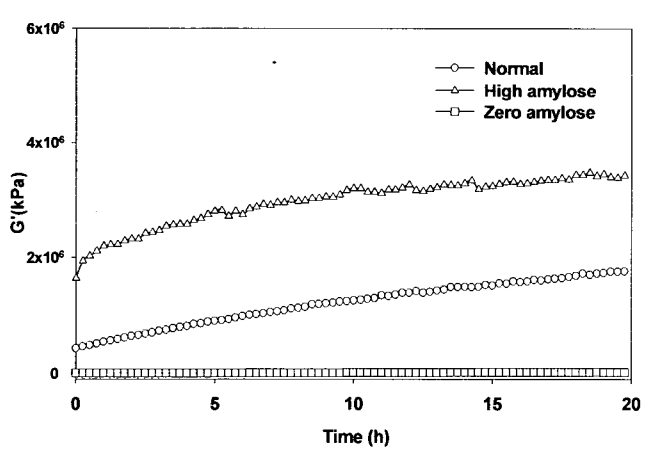

Fig. 7. Time development of storage modulus (G′) for normal, high amylose, and zero amylose barley starch gels (40%, w/w) examined at 8°C.

Fig. 6 shows the retrogradation kinetic mechanism of barley starches after gelatinization, as traced by DSC. Various types of barley starches exhibited significantly different retrogradation properties. Zero amylose starch showed very slow retrogradation rates, up to 7 days of storage, while high amylose starch displayed the most rapid retrogradation during the entire storage period.

These differences can be primarily due to the different amylose content in barley starches. After gelatinization of starches, the rapid association of exuded amylose polymers appeared to facilitate the initial gelation of normal and high amylose starch samples, which enhanced the further thickening and rigidity development of gels during storage. Another possibility for the lower retrogradation rate of zero amylose starch might be due to the fact that the higher M_w of its amylopectin polymer lowers chain mobility of this polymer (Table 1). Moreover, differences in the chain length of linear amylopectin branches in barley starches with variable amylose content (Fig. 3) might significantly affect the retrogradation properties of the starches.

Slow retrogradation of zero amylose starch was also confirmed by rheological evaluations. Fig. 7 shows the kinetics of storage modulus (G′) development for various starches during 20 h storage. Increases in the rigidity of zero amylose starch gel were minimal compared to those of normal and high amylose barley starches, which indicated an excellent storage stability of zero amylose starch sample.

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