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Molecular characteristics of barley starches with variable amylose content

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Abstract

The granular and molecular characteristics of four types of hulless barley starches (normal, high amylose, waxy and zero amylose) were investigated by particle size analysis and size exclusion chromatography (SEC) with light scattering detection. Although bimodal size distribution of granules was observed for normal, waxy and zero amylose starches, the proportion of large to small granules for each type of starch differed substantially. The granular size distribution of high amylose starches was unimodal, showing the highest proportion of small granule (3 μm). For the intact starch molecules, molecular weights of high (amylopectin) and low (amylose) M_w fractions were in the range from 136×10^6 to 305×10^6 and from 2.73×10^6 to 5.67×10^6, respectively. The lowest M_w values were observed for amylopectin in high amylose starches. A good correlation ($r^2 = 0.96$) between M_w and R_g of all amylopectins was found. After debranching of starch samples, significantly lower M_w values were observed for amylose, indicating the existence of branches in amylose molecules. Debranched amylopectins exhibited trimodal distributions of long B, intermediate B and short B + A chains. The longest amylopectin branches were found in high amylose barley starches.

Keywords: Barley starch; Molecular weight; Amylose; Amylopectin

1. Introduction

The ever growing interest in barley as a component of food systems has stemmed largely from the potential health benefits of β-glucans, a group of minor non-starch polysaccharides, present in barley grain. The other abundant group of barley polysaccharides, starch polymers, has also attracted scientific attention, although not as extensive as, for instance, corn, potato or wheat starches. Some of the recently developed hulless barley genotypes contain starches with a broad range of amylose content, varying between 0 and 40% (Izydorczyk, Storsley, Labossiere, MacGregor & Rossnagel, 2000). Since the amylose content is known to affect various functional properties, such as gelatinization, pasting, and swelling, variability in amylose level predisposes barley starches for a wide range of applications in food production. Barley starches contain both small and large granules, and a bimodal distribution of starch granules was reported by Jane, Kasemsuwan, Leas, Zobel and Robyt (1994), MacGregor and Fincher (1993) and Morrison, Scott and Karkalas (1986). The small and large starch granules can be fractionated by decantation (Takeda, Suzuki & Hizukuri, 1988), and by pin-milling and air-classification (Vasanthan & Bhatty, 1995) techniques. Recent studies have shown, however, that there are no significant difference in the molecular structure of starch polymers from small and large granules (Tang, Ando, Watanabe, Takeda & Mitsunaga, 1999; Vasanthan & Bhatty, 1996).

The length and distribution of linear branch chains in barley amylopectin can be typically determined by enzymic hydrolysis of the branching points (α-(1 → 6) linkages), followed by either gel permeation chromatography (GPC) or high performance anion-exchange chromatography (HPAEC) combined with an enzyme reactor and a pulse amperometric detector (ENZ-PAD). Tester, South, Morrison and Elluis (1991) reported no variations in chain lengths of debranched amylopectins from normal, waxy and high amylose starches, as measured by GPC on Sepharose CL 6B. Song and Jane (2000), using HPAEC-ENZ-PAD, also observed no difference in the trimodal chain profiles and chain lengths of debranched amylopectins from normal, waxy and high amylose barley starches, but showed differences in the presence of chains with the highest detectable degree of polymerization (DP) values. They found that the highest detectable DP values of normal, waxy and two types of high amylose debranched amylopectins were 82, 67, 79 and 78, respectively. Salomonsson and Sundberg (1994), on

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the other hand, reported a bimodal distributions of debranched amylopectins and found that the average chain lengths of high amylose debranched amylopectin chains was five glucose units longer than those of normal and waxy amylopectin chains. The molecular weights (M_w) of barley starch polymers, amylopectin and amylose, have not yet been accurately measured.

Because of their availability and unique characteristics, barley starches may have a potential for providing useful material for targeted food products. More thorough and systematic studies, however, are needed to fully elucidate the molecular structure of barley starches and explain the origin of their physical and functional properties. In this study, barley starches were carefully extracted from several barley genotypes with a wide range of amylose content, and the molecular structure of amylose and amylopectin polymers was examined.

2. Materials and methods

2.1. Materials

Four genotypes of hulless barley starches — normal, high amylose, waxy and zero amylose — isolated from eight varieties of barleys, were assessed. One variety, Falcon, obtained from James Farms Ltd. (Winnipeg, MB), was grown in 1998 in Manitoba, Canada. The others, SB90354, 92-55-06-54, 92-55-06-48, CDC Candle, SR 93139,SB94792 and SB94794, were grown in 1997 in Saskatchewan, Canada, at the Crop Development Centre, University of Saskatchewan, Saskatoon, Canada.

2.2. Chemical analyses

The starch content in barley samples was measured enzymatically using the total starch assay kit (Megazyme, Ireland). The results are reported on a dry weight basis. Amylose content in de-fatted barley starches was determined by potentiometric titration (Schoch, 1964). Barley samples were de-fatted by extraction in the Soxhlet apparatus with 3:1 (v/v) *n*-propanol: water for 12 h prior to the amylose determination.

2.3. Isolation of starch

The starch isolation procedure used in this study was based on the methods of Morrison, Milligan and Azudin (1984), South and Morrison (1990) and Sulaiman and Morrison (1990). Two additional enzymes, lichenase and β-xylanase were applied to disrupt the cell walls and release starch granules, thereby enhancing the quantity of isolated starch. Coarsely grounded barley kernels (10 g) were steeped in 0.02N HCl (100 ml) solution and swirled gently overnight at 4°C. After neutralization with 0.2N NaOH and centrifuging at 4000 g, the precipitate was scraped into a mortar with 30 ml of 0.1 M Tris-HCl buffer containing 0.5% $NaHSO_3$ (pH 7.0), followed by enzyme treatments, proteinase K (15 U/g barley, Roche Diagnostics Ltd. Canada), lichenase (2 U/g barley, Megazyme) and β-xylanase (8 U/g barley, Megazyme). The samples were digested overnight in a Neslab EX-700 shaking water bath (shaking speed = 1) at 25°C. After incubation, the contents were sieved on a 75 μm (#200) sieve, followed by centrifuging (28,000 g, 10 min) the slurries. The precipitate was resuspended with 4 ml of water and then layered over 100 ml of 80% CsCl solution, followed by centrifuging at 28,000 g for 30 min. After centrifuging, the starch pellet was washed with water and filtered on a 0.45 μm membrane (HVLP). Approx. 10 ml of acetone was filtered through the starch to remove remaining water. The starch was allowed to air-dry overnight.

2.4. Size distribution of starch granules

Granule size distribution was determined using a computer assisted Olympus Image Analyzer System and CUE 2 software. The purified starch (20 mg) was dispersed thoroughly in 500 μL of 20 times diluted iodine solution, followed by centrifuging shortly at 4000 xg. The residue was spread on a slide glass with two drops of water containing 0.1% Tween, and then measured by an Olympus microscope through a CCD video camera. At least five random microscope fields were measured per sample.

2.5. Determination of weight average molecular weight (M_w) of starch polymers

2.5.1. Solubilization of starch

Granular starch was gelatinized and then purified with 90% DMSO and alcohol precipitation, as stipulated by the method of Jane and Chen (1992). The purified starch (7 mg) was steeped in ethyl-alcohol (0.1 ml), redissolved in 1N NaOH (1 ml), diluted with water (8 ml) and neutralized with 1N HCl. The starch solution was autoclaved for 20 min (121°C), filtered through a 3.0 μm cellulose acetate membrane, and then injected into HPSEC-MALLS-RI system.

2.5.2. Preparation of debranched starch

The starch solution (3 ml), dissolved as above, was incubated with isoamylase (500 units) in 1 ml of acetate buffer (0.1 M, pH 3.5) for 24 h at 40°C (Ramesh, Mitchell, Jumel & Harding, 1999a). After incubation with the enzyme, the digested starch solution was neutralized with 1N NaOH, heated in a boiling water bath for 5 min to stop enzyme activity, filtered through 0.45 μm membrane, and finally injected into HPSEC-MALLS-RI system. The profiles of debranched zero amylose waxy starch were used to evaluate the completeness of debranching with isoamylase. After 24 h of incubation with the enzyme, a complete hydrolysis of amylopectin (zero amylose starch) was verified, as indicated by the lack of any high molecular weight material in the elution profile.

Table 1
Starch and amylose content in various genotypes of barley

Barley	Starch content[a] % (w/w)	Starch yield[b] (%)	Amylose content[c] (%)
Normal			
Falcon	62.0 ± 0.4	62.8	23.7
SB90354	58.7 ± 0.5	58.1	24.3
High amylose			
92-55-06-54	53.0 ± 0.5	55.8	41.5
92-55-06-48	53.7 ± 0.4	54.4	41.9
Waxy			
CDC Candle	56.0 ± 0.6	58.2	4.2
SR93139	55.5 ± 0.4	56.0	5.8
Zero amylose			
SB92792	53.5 ± 0.4	56.1	0
CDC Alamo	52.7 ± 0.5	55.5	

[a] Results reported on a dry weight basis $n = 2 \pm$ SD.
[b] Yield of starch obtained after isolation from whole barley.
[c] Determined by potentiometric titration with iodine.

2.5.3. HPSEC-MALLS-RI system

The calibration constants of the refractive index and multi-angle laser light scattering detectors were determined by the method of You, Fiedorowicz and Lim (1999). Normalization of the photo diodes located around the scattering cell was done using BSA. The high performance size exclusion chromatography (HPSEC) system consisted of a pump (Waters 510), an injection valve (Model 7010, Rheodyne) with a 200 μL sample loop, a guard column (TSK PWH, Tosoh Corp.), SEC columns, MALLS (Dawn DSP, Wyatt Technology) and RI (Waters 410). For intact starch molecules, only TSK G5000 PW column (7.8 × 600 mm, TSK PW, Tosoh Corp.) was used to determine the profiles of amylopectin and amylose; however, TSK G2500 PWXL (7.8 × 300 mm, TSK PWXL, Tosoh Corp.), TSK G3000 PWXL (7.8 × 300 mm, TSK PWXL, Tosoh Corp.) and TSK G5000 PW columns were employed to get the profiles of debranched amylopectin and amylose molecules. The columns were 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.

3. Results and discussion

3.1. Amylose content and granular size distribution

Eight starch preparations were isolated from barley varieties with varying content and composition of starch polymers (Table 1). The highest amount of starch was found in the two normal barley varieties, whereas the genotypes with atypical amylose content contained substantially less starch. This is in agreement with previous reports, which also observed that some barley genotypes with an altered ratio of amylose to amylopectin contained lower amounts of starch (Bhatty & Rossnagel, 1998; Oscarsson, Andersson, Salomonsson & Aman, 1996). It appears that the presence of waxy or high amylose genes in barley may substantially affect the carbohydrate metabolism in the grain. High amylose and waxy barleys were reported to contain significantly higher β-glucan content than their normal counterparts (Bhatty, 1999; Izydorczyk et al., 2000). Xue, Wang, Newman, Newman and Graham (1997) reported higher content of free sugars as well as β-glucans in six waxy barley isotypes.

The yield of isolated starches corresponded well with the starch content of the barley samples used, indicating that no losses, especially of small granules, occurred during the isolation process. The purity of the starch preparations was greater than 99% in all cases as revealed by the starch content of the samples (results not shown).

Barley starches are known to consist of a mixture of large, lenticular granules (10–25 μm) and smaller, irregularly shaped granules (<10 μm) (MacGregor & Fincher, 1993) distributed in a bimodal fashion. Our study confirmed those results: the granule diameter ranged from 2 to 26 μm (Fig. 1). The relative frequency of small and large granules differed, however, among the different types of starches, and the distribution did not follow the bimodal pattern in all cases. The greatest amount of large granules (≥8 μm in diameter) was found in normal starches (74.7%), whereas the smallest in high amylose starches (19.4%). The waxy and zero amylose waxy starches contained 66.4 and 43.9%

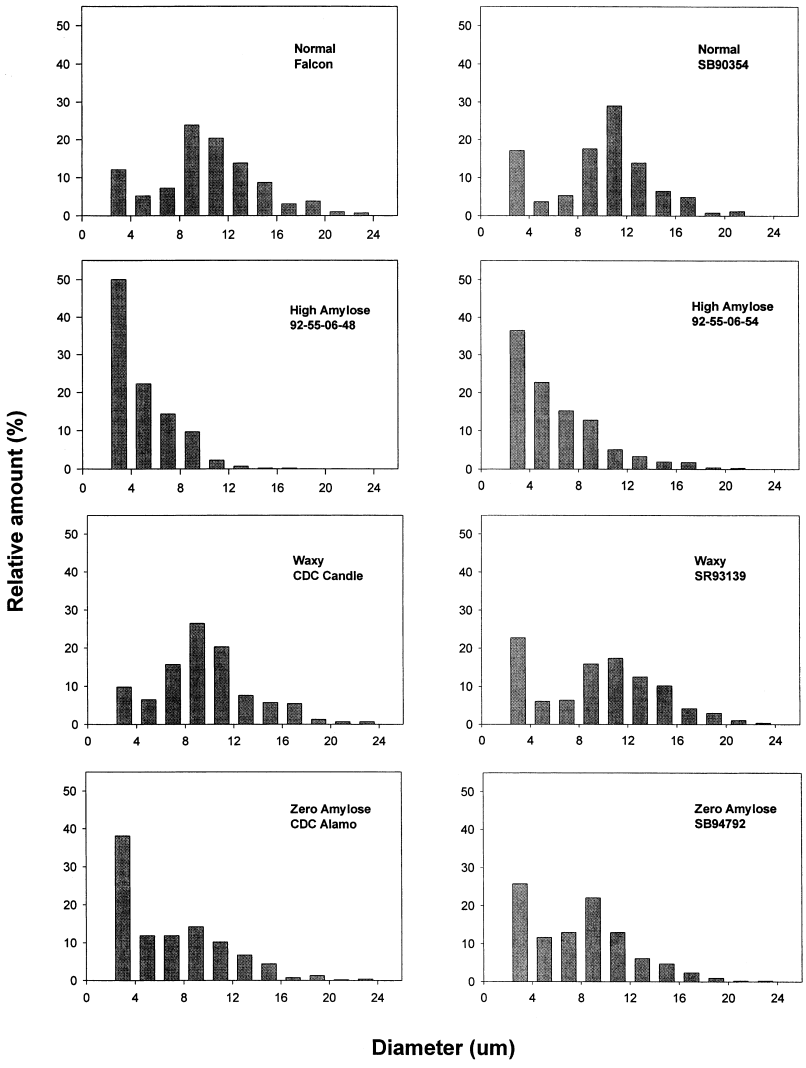

Fig. 1. Size distribution of granules in barley starches with variable amylose content.

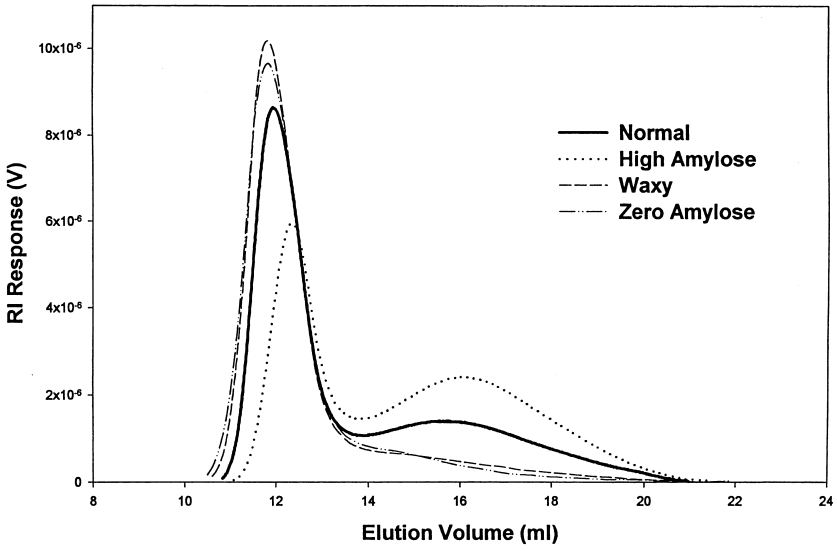

Fig. 2. HPSEC profiles of normal (Falcon), high amylose (92-55-06-48), waxy (CDC Candle), and zero amylose (CDC Alamo) barley starches.

Table 2
Weight average molecular weights (M_w), radii of gyration (R_g), and polydispersity values (M_n/M_w) of barley starches (Values followed by the same letter (column) are not significantly different ($p \leq 0.05$))

Starch type	High M_w fraction			Low M_w fraction			Recovery[a]
	$M_w \times 10^{-6}$ (g/mol)	R_g (nm)	M_w/M_n	$M_w \times 10^{-6}$ (g/mol)	R_g (nm)	M_w/M_n	(%)
Normal							
Falcon	226 ± 9.9 b	223 ± 4 d	1.90	5.67 ± 0.90	107 ± 4	1.12	86.1
SB90354	284 ± 4.2 a	240 ± 1 c	1.93	4.42 ± 0.01	98 ± 2	1.23	87.8
High amylose							
92-55-06-54	141 ± 4.9 c	172 ± 1 e	1.93	2.70 ± 0.10	64 ± 1	2.61	85.6
92-55-06-48	136 ± 6.7 c	164 ± 5 e	1.85	2.73 ± 0.10	65 ± 2	2.21	81.2
Waxy							
CDC candle	297 ± 2.8 a	249 ± 1 b	1.58	31.9 ± 7.5	141 ± 13	nd	71.6
SR93139	303 ± 11.0 a	250 ± 1 b	1.57	39.8 ± 3.3	149 ± 9		69.9
Zero amylose							
SB92792	299 ± 0.7 a	262 ± 3 a	1.59	30.5 ± 0.1	141 ± 6	nd	64.7
CDC alamo	305 ± 0.7 a	266 ± 1 a	1.60	40.7 ± 13.0	148 ± 17		71.5

[a] Mass recovery after HPSEC corresponds to the proportion (%) of injected material, recovered in the column effluent.

of the large granules, respectively. Even though the proportion of large to small granules in normal and waxy starches differed substantially, their bimodal distribution was still observed. High amylose starch granules, on the other hand, were distributed unimodally, with the highest proportion of 3 μm granules and a sharply decreasing amounts of larger granules. Some differences in the granule size distribution were observed not only among the different types of barleys but also between samples within the same starch type.

3.2. M_w and R_g of starch polymers

The elution profiles of various barley starches from the size exclusion chromatography (SEC) column are presented in Fig. 2. The chromatograms indicate a good separation of the large and small molecular weight starch components. The first, high molecular weight peak in the chromatograms, with concentration maxima at the elution volume (V_e) of approximately 12.0 ml, constitutes the majority of amylopectin fraction in each starch sample, whereas the second, low molecular weight peak, at V_e maximum about 16.0 ml, corresponds largely to amylose. The relative proportion of the two peaks gives some indication of the concentrations of amylose and amylopectin in each type of starch. However, determination of amylose content in the samples on the basis of chromatographic data (i.e. integration of peaks) would likely lead to an overestimation of amylose content because some amylopectins also elute in the lower molecular weight region, traditionally assigned to amylose. The average molecular weights of high and low molecular weight components in each starch sample are compiled in Table 2. There were significant differences in the molecular weights and radii of gyration among the high M_w polymers (amylopectins). Amylopectins from waxy and zero amylose starches had the highest average molecular weight, whereas those from high amylose starches the lowest. Interestingly, there was a good correlation ($r^2 = 0.96$) between the molecular weights of all amylopectins and their dimensions, as estimated from the radius of gyration (R_g). Fig. 3a shows the relationship between the molecular weights and the elution volume. Some differences in the slopes of M_w dependence on V_e among amylopectins from different barley types were observed only for the higher V_e values. This could indicate some branching differences between amylopectins from waxy versus normal or high amylose starches. But, it is also conceivable that some of the linear polymers (i.e. amylose-like) start to elute in this region and that they contribute to the change of slope of the M_w versus V_e relationship. In general, our results revealed significant differences in the M_w and R_g but rather small differences in the conformation of amylopectins from different types of barley starches.

Polydispersity values (M_w/M_n) of amylopectins from waxy and zero amylose starches were lower than those from normal and high amylose starches. The broad distribution of molecular masses for amylopectins in normal and high amylose starches can be clearly seen in Fig. 4, where differential weight fraction is plotted as a function of molecular mass. Amylopectins from normal and high amylose starches exhibited a broad range of polymer populations with both higher and lower molar masses than amylopectins from waxy barley starches.

The molar masses and dimensions of barley amylopectin found in this study fall in the range reported for amylopectin of other botanical origin. Bello-Perez, Roger, Baud and Colonna (1998) reported molecular mass of $2.2 \pm 0.2 \times 10^8$ g/mol for waxy maize amylopectin, $1.9 \pm 0.3 \times 10^8$ g/mol for normal maize, and $3.5 \pm 0.2 \times 10^7$ g/mol for high amylose maize. Much lower values were communicated, however,

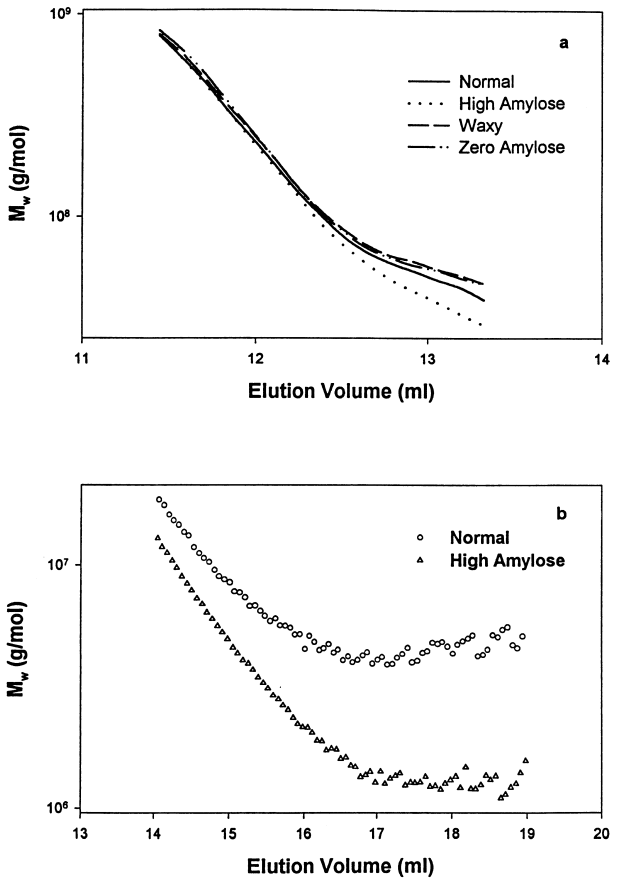

Fig. 3. Relative molecular weight vs elution volume for high (a) and low (b) molecular weight fractions in normal (Falcon), high amylose (92-55-06-48), waxy (CDC Candle), and zero amylose (CDC Alamo) barley starches.

by Fishman and Hoagland (1994) for waxy ($2.0 \pm 0.2 \times 10^7$ g/mol), normal ($1.3 \pm 0.2 \times 10^7$ g/mol), and Amy V ($8.0 \pm 0.4 \times 10^6$ g/mol) maize starches. These discrepancies might be due to different solubilization methods used in various studies. It is well known that in order to determine properly the molecular weight of starches, complete solubilization of starch polymers, amylose and amylopectin, in an appropriate solvent must be ensured. Concurrently, the degradation of starch polymers must be avoided. Degradation or incomplete solubilization of starch polymers may lead to underestimation of true molecular weight of starch polymers. On the other hand, incomplete disaggregation would lead to overestimation of the molecular weights. Due to the unique molecular structure and not yet sufficiently understood physico-chemical behavior of starches in solution, complete solubilization of starch possesses a problem. Various approaches to achieve starch dissolution have been attempted ranging from mild to more severe treatments. Fishman and Hoagland (1994) and Fishman, Rodriguez and Chau (1996) dissolved starch in water by microwave heating (80 s) in a high pressure vessel. Recently Bello-Perez et al. (1998) reported that the optimum conditions for starch solubilization involved pre-treatment of samples with dimethyl sulphoxide and dissolution by microwave heating for 35 s in a high pressure vessel (maximum temperature 143°C). Many workers have attempted to solubilize starch by autoclaving under various conditions. Aberle, Burchard, Vorwerg and Radosta (1994)reported that optimum autoclave temperature for molecular dissolution without degradation varied from 135–155°C depending on the origin of starch. Hanselmann, Ehrat and Widmer (1995) and Hanselmann, Burchard, Ehrat and Widmer (1996) observed a decrease in molecular weight of waxy corn starch when the samples were autoclaved at 175°C for longer than 20 min. However, the authors postulated also that the higher molecular weight obtained after shorter autoclaving period might be due to the fact that a heating period of 20 min was not sufficiently long to dissolve starch completely and, therefore, the high molecular weight corresponds to large aggregates of starch polymers. You and

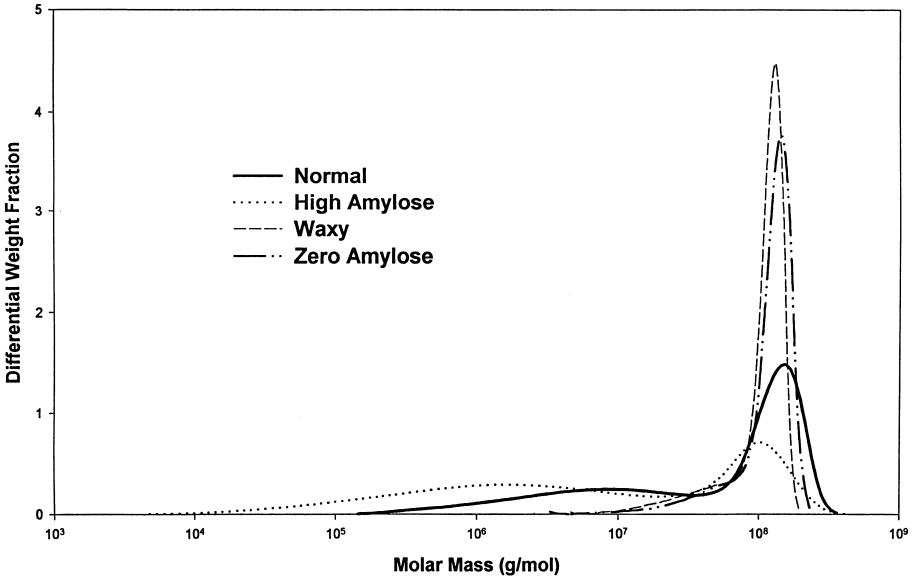

Fig. 4. Molar mass distribution in normal (Falcon), high amylose (92-55-06-48), waxy (CDC Candle), and zero amylose (CDC Alamo) barley starches.

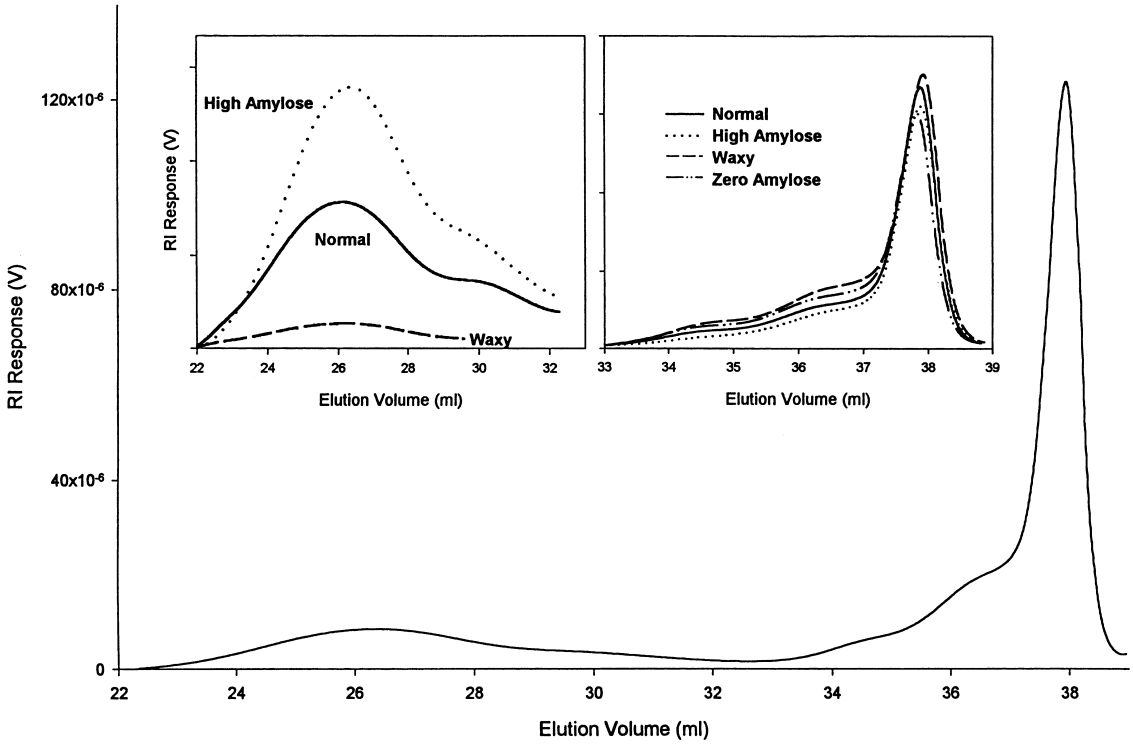

Fig. 5. HPSEC profiles of normal (Falcon), high amylose (92-55-06-48), waxy (CDC Candle), and zero amylose (CDC Alamo) barley starches after debranching with isoamylase.

Lim (2000) reported that autoclaving (121°C, 20 min) or microwave heating (35 s) provided better HPSEC recovery and higher M_w for starch molecules than simple dissolution in hot water. Our studies concurred with the previously published data; lower M_w and lower recoveries were obtained when the barley starches were not autoclaved (data not shown).

Although the elution volumes of the low molecular weight peaks assigned to amylose in normal and high amylose starches showed no prominent differences (Fig. 2), the calculated molecular weights were significantly different, with the average M_w of amyloses in the two normal starches almost twice as high as those of the two high amylose starches (Table 2). The differences in the relationship between M_w versus V_e are also shown in Fig. 3b; at the same elution volume, amylose from normal starch displayed greater M_w than high amylose samples. Normally, different slopes of log M_w versus V_e might indicate variations in the conformation of polymers. In this case, however, the observed differences in the molecular weight and conformation of the low M_w material might arise from the fact that some of branched material (i.e. amylopectin) eluted in this region traditionally assigned to amylose. This was apparent from the elution profiles of waxy and zero amylose starches, which showed a small but definite population of polymers eluting in the volumes assigned normally to amylose, despite very small content or even absence of amylose fraction in these particular samples. The molecular weights and radii of gyration of these materials in waxy samples remained, however, very high (Table 2). Accurate determination of molecular characteristics of amylose may, therefore, be rendered problematic by the presence of branched material eluting in the same volumes.

Substantial differences in polydispersity among the low molecular weight fractions were observed (Table 2). Polydispersity of high amylose starches was much greater than normal and waxy starches (Table 2 and Fig. 3). Of the four types of barley starches, high amylose starches contained polymers with the broadest range of molecular masses (Fig. 3).

The overall mass recovery after HPSEC ranged from approximately 65% to 87% and appeared to be dependent on the amylose/amylopectin ratio in the samples (Table 2). In general, normal and high amylose starches showed higher HPSEC recovery than waxy and zero amylose starches. The higher recovery of samples containing a higher proportion of amylose has been reported previously (Bello-Perez et al., 1998; Yokoyama, Renner-Nantz & Shoemaker, 1998). Difficulties associated with total solubilization of starch polymers and non-quantitative elution of amylopectin, in particular, have been blamed for low mass recovery after SEC of starches.

3.3. Molecular characteristics of debranched starches

3.3.1. Debranched amylose

The elution profiles of the starch samples treated with the debranching enzyme are shown in Fig. 5. In addition to the low molecular weight material (V_e: 33–39 ml) originating

Table 3
Weight average molecular weights (M_w), radii of gyration (R_g), and polydispersity values (M_n/M_w) of amylose after debranching of starch samples with isoamylase (Values followed by the same letter (column) are not significantly different ($p \leq 0.05$))

Sample	Fraction I			Fraction II			Amylose content[a] (%)
	$M_w \times 10^{-3}$ (g/mol)	R_g	M_w/M_n	$M_w \times 10^{-3}$ (g/mol)	R_g	M_w/M_n	
Normal							
Falcon	796 ± 42.4 ab	45.8 ± 3.9 ab	1.79	100 ± 7.0 a	32.1 ± 1.6 a	1.05	15.1
SB90354	861 ± 36.0 a	46.8 ± 1.0 ab	1.7	101 ± 5.0 a	26.1 ± 1.8 a	1.15	15.9
High amylose							
92-55-06-54	782 ± 59.0 ab	43.1 ± 1.8 ab	1.5	97.9 ± 2.6 a	21.1 ± 0.6 a	1.2	22.6
92-55-06-48	695 ± 68.6 bc	41.6 ± 3.0 ab	1.59	88.3 ± 1.5 a	26.7 ± 5.8 a	1.16	24.2
Waxy							
CDC Candle	821 ± 29.0 ab	50.5 ± 1.2 a	1.95	nd[b]	nd		2.2
SR93139	591 ± 3.5 c	37.5 ± 4.5 b	1.9				1.7

[a] Determined from chromatography data.
[b] nd, not detected.

from debranched amylopectin, the high amylose, normal, and waxy starches also showed a high molecular weight peak (V_e: 22–32 ml) presumed to be the amylose fraction. The presence of at least two populations of linear polymers in high amylose and normal starches can be inferred from the shapes of the eluting peaks. The average molecular weights of the two populations, referred to as Fraction I and II, are compiled in Table 3. The molecular weights of the debranched amylose fractions were significantly lower than those determined from the elution of intact starches (Table 2). Non symmetrical shapes of the peaks as well as rather low molecular weights of amylose fractions obtained after enzymic treatments of starch samples suggest a certain degree of branching in amylose fractions of barley starches. Interestingly, there were some differences between M_w and R_g of debranched amylose fractions (Table 3) in normal and high amylose starches but not of the same magnitudes as in intact materials (Table 2). These results confirmed our earlier supposition that the differences in molecular characteristics of amyloses inferred from the SEC of intact starches may have been intensified by the presence of amylopectin in the low M_w peak traditionally assigned to amylose.

The content of amylose fraction determined from the integration of high (amylose) and low (debranched amylopectin) M_w peaks is also presented in Table 3. These values are not in good agreement with the total amylose content measured by the iodine potentiometric method (Table 1). It has been suggested the long B chains of amylopectin molecules might also bind some iodine and, therefore, lead to an overestimation of the true amylose content in starches (Ramesh et al., 1999a,b; Song & Jane, 2000; Takeda and

Table 4
Molecular characteristics of amylopectin linear branches obtained after debranching of starch samples with isoamylase (Values followed by the same letter (column) are not significantly different ($p \leq 0.05$))

Sample	B chains (long)		B chains (intermediate)		B (short) + A chains	
	$M_w \times 10^{-3}$ (g/mol)	Relative amount[a] (%)	$M_w \times 10^{-3}$ (g/mol)	Relative amount (%)	$M_w \times 10^{-3}$ (g/mol)	Relative amount (%)
Normal						
Falcon	17.7 ± 1.2 ab	8.3	5.5 ± 0.3 b	23.4	1.5 ± 0.5 a	68.5
SB90354	16.1 ± 0.8 b	8.7	5.3 ± 0.1 b	24.4	1.5 ± 0.2 a	67.1
High amylose						
92-55-06-54	21.6 ± 0.7a	5.8	5.7 ± 0.1 ab	20	1.2 ± 0.0 a	74.2
92-55-06-48	21.3 ± 3.2 a	6.7	6.3 ± 0.5 a	20.4	1.9 ± 0.4 a	73
Waxy						
CDC candle	13.6 ± 0.1 bc	11.8	4.1 ± 0.1 c	27.1	1.4 ± 0.0 a	61.2
SR93139	11.2 ± 1.1 c	9.8	4.4 ± 0.1 c	28.5	1.1 ± 0.1 a	61.7
Zero amylose						
SB92792	9.5 ± 0.4 c	12.1	3.8 ± 0.1 c	27.5	1.2 ± 0.1 a	60.5
CDC alamo	11.1 ± 0.8 c	10.1	4.3 ± 0.0 c	29.3	1.6 ± 0.1 a	60.7

[a] % of the sum of B chains (long) + B chains (intermediate) + B (short) + A chains.

Hizukuri 1987). However, to assure the accuracy of amylose determination from the SEC of debranched starch, the quantitative elution of the polymeric material from the chromatographic column must be guaranteed.

3.3.2. Debranched amylopectin

The debranched amylopectin molecules from four types of barley starches exhibited similar profiles, all showing trimodal distributions of long B (V_e: 33–35.2 ml), intermediate B (V_e: 35.2–37.3 ml), and short chains B or A (V_e; 37.3–38.9 ml) (Fig. 5). The M_w of long B chains from the debranched amylopectins varied from 9.5×10^3 to 21.6×10^3 g/mol, which corresponds to the weight average DP values from 59 to 133 (Table 4). The highest M_w and DP values for the long B chain were observed for high amylose starch, followed by normal, waxy, and zero amylose starches. The relative amounts of the long chains in high amylose samples were, however, the lowest. These results do not confirm the results of the recent studies by Song and Jane (2000), where the distribution of linear chains was studied with HPAEC combined with PAD. They reported that normal starch contained the longest linear chains with the DP values of 82. These discrepancies may be due to the differences in the varietal origin of starches as well as to differences in the methods employed for the detection of the debranched chains.

The M_w values of the intermediate B chains ranged from 3.8×10^3 to 6.3×10^3 g/mol, which corresponds to the DP of 23–39. The intermediate B chains of high amylose starch had relatively higher DP values than those of other starches. The lowest DP values of intermediate B chains were found in waxy and zero amylose starches. Song and Jane (2000) reported no significant differences in the average length of intermediate chains from four varieties of barley starches. The M_w of short B or A chains from the debranched amylopectins were in the range of 1.1×10^3–1.9×10^3 g/mol (DP 6.8–12).

Interestingly, the molecular size (R_g) of the linear chains of amylopectins was not in good agreement with their molecular weights, indicating that R_g of the short B or A chains was greater than R_g of long and intermediate B chains. A similar observation was recently made by You et al. (1999). It is possible that the short glucan chains (DP 6-12) remain fully stretched during the chromatographic analysis, whereas the longer chains (DP $>$ 20) are capable of assuming more folded or even coiled conformation supported by numerous secondary forces. It has been well documented that polymeric chains of α-glucans have a high tendency to adopt random coil structures with helical segments in alkaline and neutral aqueous solutions (Rao, Qasba, Balaji & chandrasekaran, 1998).

4. Conclusions

Significant differences in the granule size distribution and molecular characteristics of amylose and amylopectin were found in barley starches with variable amylose content. The bimodal distribution of starch granules was observed for normal and waxy barley starches but the proportion of large and small granules in these samples differed substantially. High amylose starch granules exhibited a rather unimodal granule distribution characterized by the highest proportion of 3 μm granules. The M_w and R_g of amylopectins from waxy barleys were significantly higher than those from normal and high amylose starches. The length of some linear chains in amylopectin from high amylose samples was found to be significantly larger than in normal and waxy starches. The amylose polymers in normal and high amylose barley starches exhibited a certain degree of branching. The M_w of amylose in high amylose barley starch appeared to be lower than in normal barley starch. The substantial differences in molecular characteristics of barley starches may have significant influence on their physicochemical and functional performance. The exact nature of that influence will now have to be fully investigated.

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