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The effects of amylose content on the molecular size of amylose, and on the distribution of amylopectin chain length in maize starches

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The amylose to amylopectin ratios in six maize starch samples of differing amylose contents were measured by enzymatic debranching, followed by high performance size exclusion chromatography (HPSEC). The molecular size of amyloses, estimated by $-\log K_{wav}$, shows progressive decrease with the increase in amylose content in maize starches. The gel permeation chromatographs of the corresponding amylopectins, debranched with isoamylase, showed bimodal distributions containing long and short chains. The average chain length of amylopectin has a correlation with amylose content. The correlation coefficients between amylose content and average chain length, long chain length, weight ratio and the mole ratio of long and short chain length, were 0.97, 0.92, 0.96, 0.94 respectively. The maize starch with the highest amylose content has the lowest amylose molecular size and the longest chains, with a high ratio of long to short chains in its amylopectin fraction. Comparing the values of amylose content determined by HPSEC of starch or debranched starch with those of the iodine-complex method, we conclude that long chains of amylopectin in high amylose starches contribute significantly to apparent amylose content.

INTRODUCTION

Many unique functional properties of starch have been utilized for industrial applications. These properties are influenced by the granular and molecular structures. The weight-average chain length of the amylopectin has a close relationship with the crystalline structure and physical properties of starch granules. The amylopectin molecules of B-type starches have greater DP_n in both the long-chain fraction and the short-chain fraction than those of A type starches. The B-type starches have a smaller proportion of the short chain-fraction than do A-type starches (Hizukuri, 1985). Yuan *et al.* (1993) studied the effect of amylopectin fine structure (chain length and A:B chain ratio) on the thermal behavior of maize starch granules. They found that an increased proportion of longer chains in amylopectins causes high ΔH and T_{max} values for both gelatinization and retrogradation.

Studies on reconstituted maize starches show that amylopectin branch chain length strongly affects paste properties, which include viscosity, gel strength and light transmittance. The long branch chain amylopectin and intermediate sized amylose produce the greatest synergistic effect on viscosity. (Jane and Chen, 1992).The chain length or molecular weight of amylose is related to the tendency to retrograde (Suzuki *et al.*, 1981; Takeda *et al.*, 1983).

On the other hand, amylose content also strongly affects the structure and properties of starch granules. Gidley and Bociek (1985) found by NMR measurements that double helical content increased with decreased amylose content. Small-angle X-ray scattering results show that amylose can disrupt the structural order within the amylopectin crystallites (Jenkins and Donald, 1995). The changes in enthalpies of gelatinization and water-binding capacity of starch granules are strongly influenced by their amylose contents (Russel, 1987; Stevens and Elton, 1971; Wootton and Bamunuarachchi, 1978). In addition, Reddy *et al.* (1994) found that the amylose content has a close correlation with the paste viscosity in some

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concentration areas. The starch granules with higher amylose contents were relatively rigid, elastic and strong, while those with lower amylose content were soft, inelastic and weak, and broke down more easily.

The above studies all show that amylose content and amylopectin fine structure determine the properties of starch granules. Therefore, efforts were made here to characterize amylose contents and amylose molecular sizes and the distributions of chain length in amylopectin for maize starches using HPLC methods.

EXPERIMENTAL

Materials

The samples of six maize starches (MS) with different amylose contents used in this study were kindly provided by Starch Australasia Ltd. Their apparent amylose contents of 0% (waxy maize(WMS)), 28% (MSA), 40% (MSB), 56% (MSC), 65% (MSD), and 84%(MSE) were determined by the amperometric iodine binding method by Professor N. K. Matheson. Isoamylase was obtained from Sigma Chemical Co., and β-amylase from Megzyme, Australia. PL-gel size exclusion columns were obtained from Polymer Laboratories Inc., Amherst, MA, and a Jordi Gel 1000A GBR column (300 × 7.8 mm) was obtained from Alltech Associates Inc., Deerfield, IL.

The determination of wavelength (max) and measurement of blue value (iodine complex)

The sample of starch, amylopectin (5 mg) or amylose (2 mg)was dissolved in 8 ml 90% dimethyl sulfoxide with 0.006 M iodine overnight at room temperature, followed by vigorous vortex mixing. The dissolved sample (1 ml) was mixed with distilled water (8 ml). The blank was treated as above without any starch. After 30 min development, the absorption curves of starch–iodine complexes vs. wavelength were recorded using an Hitachi U-3200 Vis/UV spectrophotometer in the range 250–800 nm. The absorbance at $\lambda = 600$ nm was measured using a 10 mm cell. The blue value was expressed as 4A/C (mg/100 ml)(Gilbert and Spragg, 1964).

Fractionation of starches with 1-butanol

The dimethyl sulfoxide-dissolved starch samples were fractionated by complexation with 1-butanol. Maize starch (1.5 g) was defatted by refluxing with 95% ethanol, followed by three replications of dissolution and precipitation with 90% DMSO under nitrogen. The fractionation of starches was achieved by addition of 5 volumes of 10% butanol with 0.1% NaCl solution, stirring at 70°C for 1 h, followed by cooling to room temperature and centrifugation (6000 *g*, 20 min, 4°C). The resulting precipitate was redissolved in water (70°C) under a nitrogen stream with stirring for 30 min. Butanol (10%, 300–500 ml) in 0.1% NaCl solution were added, then the mixture was stirred for 10 min, and cooled slowly to room temperature. This step was repeated several times. The amylose, nonbranched high molecular weight glucan and long-chain branched glucan precipitated, whereas the short-chain branched components and low molecular weight nonbranched glucans remained in the supernatant solution. The solution was stored for 24 h (4°C). The precipitate was centrifuged (6000 *g*, 20 min, 4°C), washed with 95% ethanol, acetone, and ether under suction and dried *in vacuo* at room temperature. The noncomplexing material in the supernatant was precipitated by adding 3 volumes of 95% ethanol, and stored overnight at 4°C. The resulting precipitate was centrifuged, washed with 95% ethanol, acetone, and ether under suction, and dried *in vacuo*.

Determination of the distribution of amylose molecular size by HPSEC

The distribution of amylose molecular size was performed using a system consisting of a pump (M-45, Waters Associates Inc., Milford, MA), an injector (U6A, Waters), two PL-gel mixed 10 μm size-exclusion columns (30 cm × 7.5 mm) (Polymer Laboratories Inc., Amherst, MA) and a differential refractometer. RI detector signals were directed to a D/A converter and analyzed using Baseline 810 software (Millipore Waters) on an NEC computer.

To amylose samples (15–30 mg) in a 10 ml screw-top vial was added 90% dimethyl sulfoxide containing 0.03 M sodium nitrate (4 ml). The sealed vials were heated (75°C, 18 h) in an air oven to dissolve the starch. After the solution was cooled to room temperature, the samples were filtered before chromatographic analysis.The mobile phase was 90% dimethyl sulfoxide containing 0.03 M $NaNO_3$, which had been filtered through a 0.45μm nylon membrane and degassed under vacuum. The columns and detector were maintained at 45° and 50°C respectively. The sample volume was 100 μl. The columns were eluted at a flow rate of 0.6 ml/min (approximate pressure 0.6–0.8 MPa). The refractive index detector sensitivity (8) and scale factor (20) were maintained at a constant setting for all determinations. The columns were calibrated using dextran standards (molecular weight 10 000, 40 000, 70 000, 500 000, 2 000 000 obtained from Pharmacia Fine Chemicals, Piscatamay, NJ). The log molecular weight vs. elution time plot for the dextrans was linear ($r = 0.995$). The weight-average $DP(DP_w)$ and the number average $DP(DP_n)$ were reported by computer. These measurements were repeated independently at least three times per sample. The

average values are shown in Table 1. The molecular size of amylose was also estimated from $-\log K_{wav}$, which is approximately linearly related to molecular size for each column (Tao and Matheson, 1993). K can be calculated from the following equation:

$$-\log K_{wav} = -\log(V_{ew} - V_o)/(V_t - V_o)$$

where V_t is the total volume of the column, V_o is the void volume; V_{ew} is the weighted-average elution volume, and can be calculated from the following equation:

$$V_{ew} = \sum V_i A_i / \sum A_i.$$

Here, A_i and V_i are the peak area of the RI detector response and the elution volume at any time. Waxy maize starch and glucose were used to determine the void volume and total volume of the column respectively.

Enzymic debranching of starches

To maize starch (90 mg) was added 90% DMSO (0.5 ml). The solution was kept at 80°C for 4 h to give a completely clear solution. Ammonium acetate and acetic acid buffer (0.2 M, 9.5 ml, pH 3.5) containing 0.02% sodium azide, to prevent bacterial growth, was added, followed by isoamylase (50 μl, 140 units/ml). The solution was incubated in a water bath at 40°C. The solutions were clear. Only high amylose starches such as MSE and MSD had some gel-like character. This may have been due to aggregation and retrogradation of amylose and long-chain debranched amylopectin (Bradbury and Bello, 1993). The gel-like precipitate can be suspended or dissolved by shaking the tube. Hydrolysis was monitored by the Nelson reducing sugar method (Nelson, 1944). The per cent hydrolysis was calculated as reducing capacity (glucose equivalents) / polysaccharide in the digest (glucose equivalents)×100. Under the above conditions, the amylopectin was debranched completely in 48 h. The reaction was stopped by boiling for 5 min to inactivate the enzyme, adding 2.5 M KOH (0.2 ml) to the hydrolysate solution (0.8 ml) and mixing well. After filtration (glass-fibre paper (Whatman GF/A)), the filtrate was subjected to HPLC analysis. The contents of each fraction were calculated from the elution areas.

Debranching of amylopectin

Amylopectin was debranched by treating starch with isoamylase as described above, without pre-fractionation of the amylopectin, according to the method of Hizukuri (1985). After debranching with isoamylase, the hydrolysate (1.8 ml) was heated in boiling water for 10 min to inactivate the enzyme, mixed with *n*-butanol (0.2 ml), further incubated at 40°C for 1–1.5 h to precipitate the 1-butanol complex of the long-chain amylose, then centrifuged (1000 rpm) for 5 min. The supernatant solution was decanted, filtered through glass-fibre filter paper, and subjected to HPLC analysis.

HPLC analysis

The debranched sample solution (20 ml) was injected on to a Jordi GEL 1000A column fitted with a guard column. The mobile phase was 0.25 M KOH prefiltered (0.45 μm membrane) and degassed under vacuum. The flow rate was 0.5 ml/min. The detector response was recorded by differential refractometer and analyzed using a SMAD Data system software (Morgan Kennedy Research) linked to a Macintosh computer. Standard pullulan (Shodex p80) solutions (0.5 mg/ml in 0.25 M KOH) were injected into the HPLC system under the conditions described to generate a calibration curve.

Data processing

The molecular weight at any elution volume or elution time can be calculated by use of an equation obtained from the standard curve of pullulans of known molecular weight (p-5,p-10,p-20,p-50,) and dp6-7 from partially hydrolyzed starch. From the software, a set of data including elution time and RI detector response, was recorded for each chromatogram. The weight-

Table 1. Structures and properties of amyloses from several maize starch sources

Properties	MSA	MSB	MSC	MSD	MSE
Blue value	1.36	1.34	1.35	1.39	1.40
λ_{max} (nm)	640	640	645	645	640
DP_w	2346	2192	2130	1884	1766
DP_n	873	790	740	718	662
DP_w/DP_n	2.72	2.77	2.88	2.62	2.67
β-amylolysis limit (%)	87	85	83	84	86
$-\log K_{wav}$	0.51	0.49	0.48	0.47	0.46

average molecular weight of the sample was calculated according to

$$\bar{M}_W \frac{\sum(A_i M_i)}{\sum A_i}.$$

Here, A_i is the peak area at any time, and M_i is the corresponding molecular weight at that time. The number-average molecular weight can be calculated according to

$$\bar{M}_W \frac{\sum(A_i)}{\sum A_i/M_i}.$$

It was assumed that the peak area of the refractive index detector response was proportional to the weight fraction, irrespective of molecular weight (Hizukuri and Takagi, 1984), i.e. the weight percentage of the fraction is the same as the area percentage of the fraction. The ratio of weight percentages between the short-chain fraction (F2) and the long-chain fraction (F1) was calculated by dividing the F2 area by the F1 area. Weight-average and number-average chain lengths for each peak were calculated from the corresponding weight-average molecular weight or number-average molecular weight divided by 162. Each data point was the mean of two or three experimental results. The relative mole content of each fraction was calculated from the ratio of weight percentage to $\overline{CL}$ and the mole percentage of the fraction was determined by the ratio of the relative mole content of the fraction to the sum of the relative mole contents of all fractions. The average external chain length (ECL) and the average internal chain length (ICL) were calculated from the following equations (Yun and Matheson, 1993):

$$ECL = (CL \times \beta\text{-limit}) + 2$$

$$ICL = CL - ECL - 1.$$

The determination of reducing capacity

The method of Nelson (1944) was used with slight modification. Digest (1 ml) was diluted with 4 ml water to make the concentration of carbohydrate in the range 20–100 μg/ml. This solution (1 ml) was pipetted into a pyrex test tube. The tubes were placed in a rack that maintained them from the vertical, and 1 ml copper reagent (copper A : copper B = 25:1 (v/v)) was added to each. The rack of tubes was placed in a boiling water bath. Each test tube was covered by a suitably sized marble to avoid decrease in solution volume due to evaporation. After 20 min, the rack was placed in cold water for 10 min, 1 ml arsenomolybolate reagent was added, the mixture was diluted with distilled water (7 ml) and the absorbance was read at 625 nm. The amount of reducing sugar in each tube was calculated from the standard curve prepared with 0–60 μg/ml solution of D-glucose or maltose for β-amylolysis.

Determination of β-amylolysis limits

The sample (3 mg) was dissolved in 10 ml ammonium-acetate–acetic-acid buffer (pH 4.5, 0.2 M), and hydrolyzed by adding 5 μl β-amylase (Megazyme, 1000 units/ml) at 37°C for 24 h. The solution was analyzed for the reducing activity by the Nelson method, and for total carbohydrate by the phenol–sulphuric-acid method. β-amylolysis of starch was calculated according to the following equation (Hood and Mercier, 1978):

$$\beta\text{-amylolysis \%} = [\text{reducing capacity (maltose equivalents)} / \text{total carbohydrate(glucose equivalents)}] \times 100.$$

RESULTS AND DISCUSSION

The correlation between amylose content and molecular size of amylose

The distributions of molecular size and molecular weight of amylose samples, separated from maize starches of differing amylose content, were studied by high performance size exclusion chromatography (HPSEC) methods. In order to avoid the effect of poor solubility of amylose in aqueous solution on molecular size, 90% dimethyl sulfoxide (DMSO) with 0.03 M sodium nitrate was selected as the mobile phase. Dimethyl sulfoxide is known to be a good solvent for starch, causing little degradation, and a low molecular weight electrolyte (sodium nitrate) in the mobile phase can eliminate ionic interaction between starch molecules and packing materials (Chuang and Sydor, 1987).

The elution profiles obtained by HPSEC on PL-gel mixed 10 μm size-exclusion columns are shown in Fig. 1. The single elution peak indicates no amylopectin was present. The high blue value and λ_{max} are characteristic of amylose. When amylose fractions were treated with isoamylase, there was a slight change in reducing capacity. β-amylolyses of amyloses were between 83 and 87%, suggesting a small number of α(1-6) linkages were present. The DP_w/DP_n values are variable in different samples, all showing broad molecular weight distribution. It can be seen (Fig. 1) that the distributions of the molecular sizes of five amyloses were basically symmetrical, only MSE being skewed toward the low molecular weight. The asymmetry in the ascending versus descending portion of the curves might be because the longer chain molecules have a greater extent of branching compared with the smaller ones, so that molecular size is closer to spherical (Takeda *et al.*, 1989). The very similar blue value λ_{max} and β-amylolysis limit suggest that amyloses

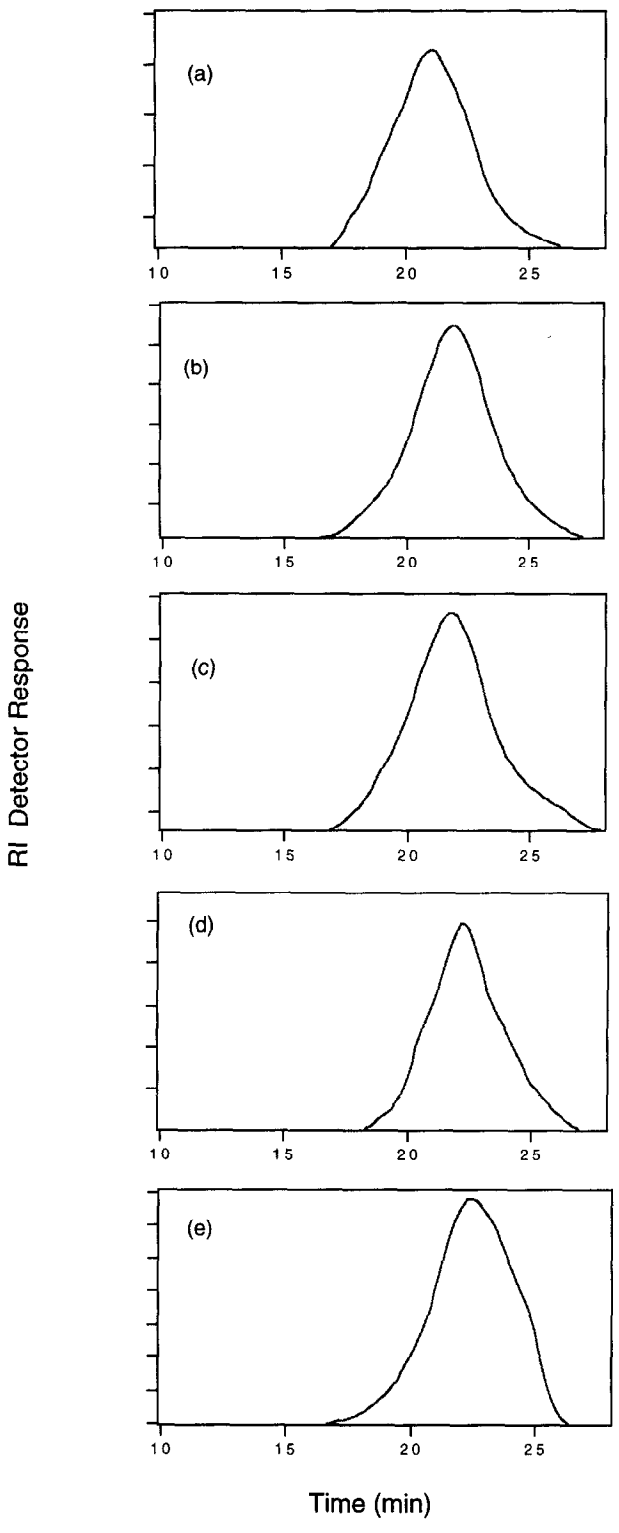

Fig. 1. HPSEC elution profiles of amyloses on the column of PL-gel: (a) MSA, (b) MSB, (c) MSC, (d) MSD, (e) MSE.

from various maize starches have similar molecular structures.

The difference in their molecular sizes is obvious. The elution profile of amylose from MSE shows a larger elution volume than from MSA. This suggests the former has a smaller molecular size than the latter. Comparing $-\log K_{wav}$ value, DP_n and DP_w of five amyloses from maize starches with different amylose contents on PL-gel (Table 1), MSA has the largest molecular size and MSE has the smallest molecular size among these amyloses, and the linear plot of $-\log K_{wav}$ vs. amylose content (Fig. 2) shows that the molecular size of amylose decreases with the increase in amylose content in maize starches. All the samples showed an apparently wide distribution of molecular sizes (Fig. 1), which were also indicated by their relative DP_w/DP_n values (Table 1). It seems that no correlation exists between the amylose content and the molecular size distribution of amylose.

The correlation between amylose content and distribution of chain lengths in amylopectin

The maize starches with different amylose content (WMS, MSA, MSB, MSC, MSD, MSE) were treated with debranching enzyme (isoamylase EC, 3.2.1.68). After removing the amylose with *n*-butanol, the supernatant liquid was fractionated by HPSEC on the Jordi Gel 1000A GBR chromatography system. The elution profiles of the amylopectins are shown in Fig. 3. The Jordi Gel 1000A GBR column has a fractionation range of 500–6000 Da and the gel is resistant to high pH (up to 1 M NaOH) and high operation pressures (to 40 MPa). Its advantage is that its fraction range is suitable for separation of amylopectin long chains and short chains. Potassium hydroxide can dissolve amylopectin completely, avoiding the tendency for long chains to retrograde, and favoring reproducible separations. It is worth

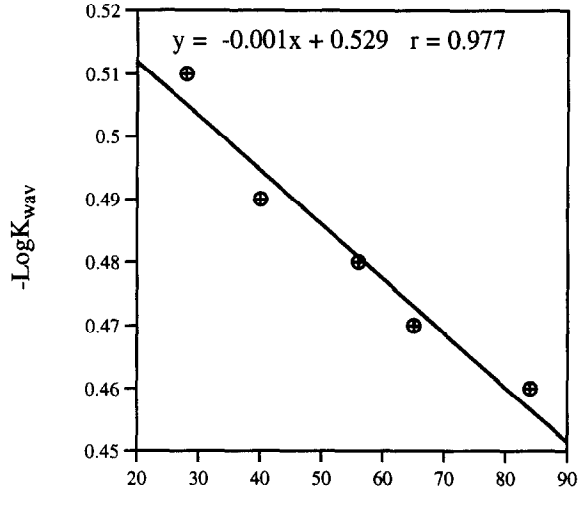

Fig. 2. The correlation between amylose content and amylose molecular size.

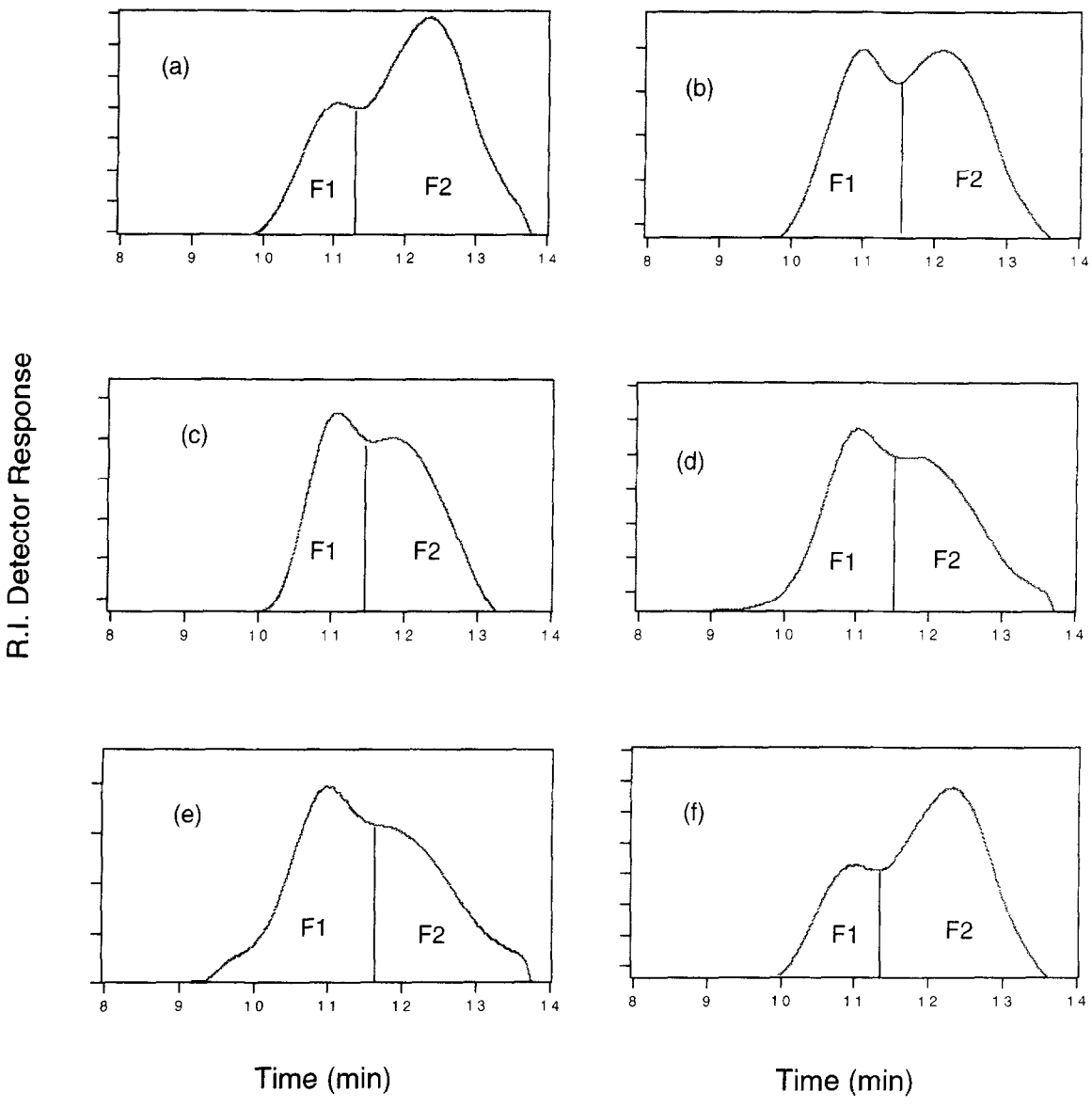

Fig. 3. HPSEC elution profiles of debranched amylopectins of maize starches with different amylose contents: (a) MSA, (b) MSB, (c) MSC, (d) MSD, (e) MSE, (f) WMS.

mentioning that maize starches with higher amylose content require a longer hydrolysis time. The broad distribution of molecular size formed initially becomes narrower with extended hydrolysis time.

After debranching by isoamylase, followed by separation by high performance size-exclusion chromatography, the chain length distribution profiles of amylopectin from maize satrches showed two overlapping peaks, designated fraction 1 (F1) and fraction 2 (F2), which varied in bimodal shape (Fig. 3). In addition, there are significant differences in average chain length, the ratio of short chain length to longer chain length (F2/F1), peak maxima values and other parameters. All the data are average values obtained from two or three duplicate experimental results. They are summarized in Table 2.

Data in Table 2 show that the amylopectins from different maize starches have similar *DP* in the short-chain fraction (F2), which suggests that amylose content has little effect on F2, but the *DP* values for F1, F2/F1, and the average chain length of the whole amylopectin show obvious differences. The average chain length of completely debranched amylopectins is in the range of DP_n 20–35. Among them MSE has the highest average chain length (35), approximately 1.5–1.7-fold those of WMS and MSA. The elution profiles of amylopectins of high amylose starches (MSC, MSD, MSE) have a much greater proportion of the high molecular weight fraction than those of low amylose starch. With increase in amylose content, the elution profile of amylopectin reveals an increase in F1 area and apparent *DP* distribution. When the apparent

Table 2. Characterization of the distribution of the chain length of amylopectin

Properties	WMS	MSA	MSB	MSC	MSD	MSE
Blue value	0.10	0.18	0.21	0.40	0.42	0.43
λmax (nm)	515	525	525	550	560	570
$\overline{CL}$ (DP_n)	20	23	25	27	30	35
β-amylolysis limit (%)[a]	50	52	53	55	58	60
ECL[b]	12	14	15	17	20	23
ICL[c]	7	8	9	9	9	11
$\overline{CL}_{F1}$	41	43	44	45	47	53
$\overline{CL}_{F1}$ (maximum)	33	40	37	36	38	39
F1 (weight %)[d]	28	30	40	43	50	56
F1 (relative mole content %)[e]	0.68	0.70	0.91	0.96	1.06	1.06
F1 (mole %)[f]	8.6	10.7	16.5	16.8	20.3	20.9
$\overline{CL}_{boundary}$	23	27	24	24	22	22
$\overline{CL}_{F2}$	10	12	13	12	12	11
$\overline{CL}_{F2}$ (maximum)	8	11	13	14	17	22
F2 (weight %)	72	70	60	57	50	44
F2 (relative mole content %)	7.20	5.83	4.61	4.75	4.17	4.0
F2 (mole %)	91.4	89.3	83.5	83.2	79.7	79.1
F2/F1 (mole % basis)	10.6	8.33	5.07	4.95	3.93	3.77
F2/F1 (weight % basis)	2.57	2.33	1.5	1.33	1.00	0.79

[a] β-Amylolysis = [reducing capacity (maltose equivalents) / total carbohydrate (glucose equivalents)] × 100.
[b] ECL = ($\overline{CL}$ × β-limit) + 2.
[c] ICL = CL − ECL − 1.
[d] F1,F2 weight % = corresponding peak area %.
[e] Relative mole percent % = weight % / $\overline{CL}_{F1}$.
[f] Mole % = F1 relative mole % / sum of relative mole % of F1 and F2.

amylose content increases from 28 to 84%, the proportion of F1 eluted increases from 30 to 56%. The large increase in F1 indicates an increase in average chain length.

Figure 4 shows the relationship between amylose content and average chain length of amylopectin. It indicates that the average chain length of amylopectin in higher amylose starches was higher than that of maize starches with lower amylose content, which is consistent with the results of Banks *et al.* (1974). The data obtained from blue values and λ_{max} measurements also provide supporting evidence. When the amylose content increases from 0 to 84%, λ_{max} increases from 515 to 570 nm and the blue value increases from 0.1 to 0.43. The significant increase of iodine-binding capacity reflects the change of average chain lengths of amylopectins.

Figure 5 shows the correlation between the amylose content and weight ratio, and the mole ratio of F2/F1 which was calculated from the ratio of the relative area of the two fractions on a weight and mole basis. The ratio of F2 to F1 reflects the degree of multiple branching of amylopectin: the higher the ratio of F2/F1, the higher the degree of branching (Biliaderis *et al.*, 1981). Fraction 1 (F1) contains the longer linear chains of debranched amylopectins (long B chains) which eluted first, and fraction 2 (F2) comprises the shorter chains of lower molecular weight, including A and short B chains of amylopectin, which eluted later. The correlation coefficients between amylose content and weight ratio as well as mole ratio of subfraction (F2/F1) were 0.96 and 0.94 respectively, implying that, with the increase in amylose content, amylopectin has a higher proportion of longer chain fraction and a relatively lower degree of branching. The results of β-amylolysis also support this conclusion because the extent of β-amylolysis was progressive accompanying increased amylose content.

The amylose content in maize starch not only strongly affects average chain length but also affects the average chain length of F1 in the corresponding amylopectin. The average chain lengths of F1 differed significantly among the maize starches with different amylose content, with waxy maize having the shortest $\overline{CL}_{F1}$(41) and MSE having the longest $\overline{CL}_{F2}$(53) (cf. Table 2). The amylose content and F1 chain length are

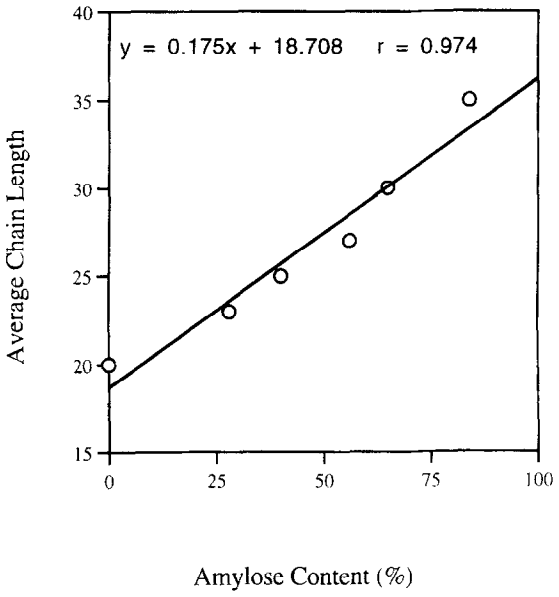

Fig. 4. The correlation between amylose and the average chain length of amylopectin.

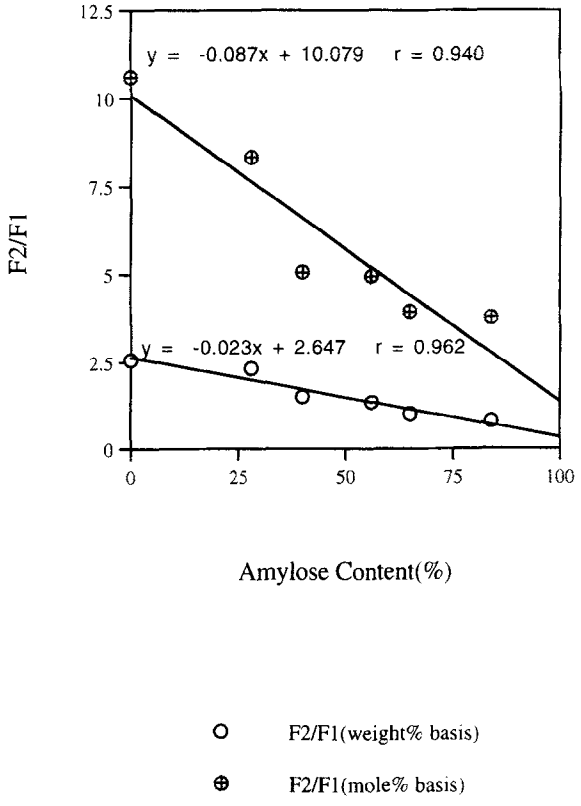

Fig. 5. Relationship between amylose content and the weight ratio and mole ratio of subfraction (F2/F1).

linearly related ($r = 0.92$) (Fig. 6). However, $\overline{CL}_{F2}$ values were not significantly different among the maize starches. This indicates that the difference of average chain length in F1 must be attributed to difference in the distributions among the relative chain lengths.

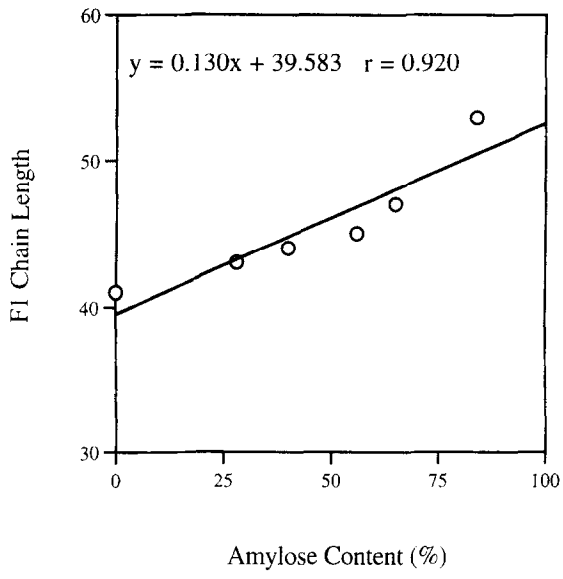

Fig. 6. The correlation between amylose content and F1 chain length.

Figure 7 reflects the relationship between the average chain length of amylopectin and the weight ratio and mole ratio of long and short chains. Their correlation coefficients are 0.935 and 0.869 respectively. Among all the elution profiles of

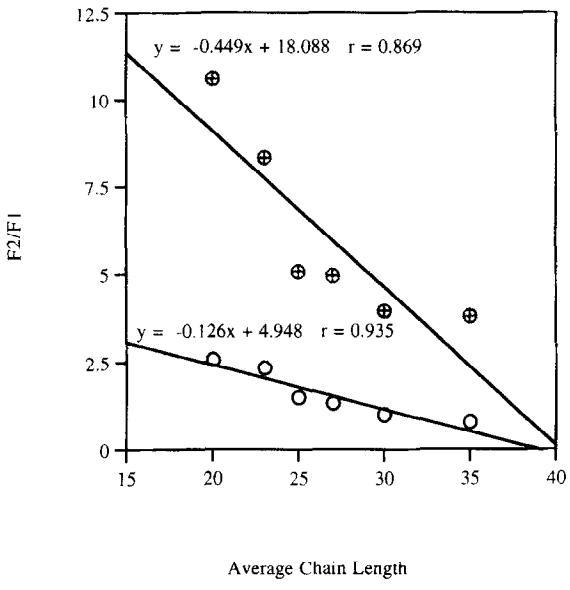

Fig. 7. Relationship between the chain lengths of amylopectin and the weight ratio and mole ratio of subfraction (F2/F1).

amylopectins, WMS has the highest ratio of F2 to F1, and MSE has the lowest ratio. For both weight per cent and mole per cent, the average chain lengths of amylopectin are negatively correlated to F2/F1, which suggests that an increase in average chain length of amylopectin is associated with an increase in long or medium chains at the expense of short chains. The maize starches with high amylose content had a low degree of branching.

Amylose content by HPLC analysis of debranched starch

Maize starches were debranched by isoamylase, then analyzed by GPC on a JORDI GEL 1000A column. Because amylose has a higher molecular weight ($1\,500\,000 > M_W > 20\,000$), it can be excluded in the void volume and separated from debranched amylopectin. The ratio of amylose and amylopectin can be calculated from the elution profiles by measurement of peak areas.

As the extent of hydrolysis increases, some white gel-like precipitation is observed. After shaking viogrously, the precipitates can be resuspended, and can be dissolved in 0.25 N KOH. The results of HPSEC in 0.25 N KOH of debranched maize starch are reproducible. Unlike normal column chromatography, HPSEC needs only a short elution time, so alkaline degradation of starch is too small to be of concern. Amylose content measured by HPSEC should thus be reliable.

Figure 8 shows the HPSEC profiles of maize starches with different amylose content. All the elution profiles exhibited three fractions clearly except WMS. F1 appeared at the void volume, and is from the amylose of the original starches, F2 and F3 originated from debranched amylopectin of maize starch; these are

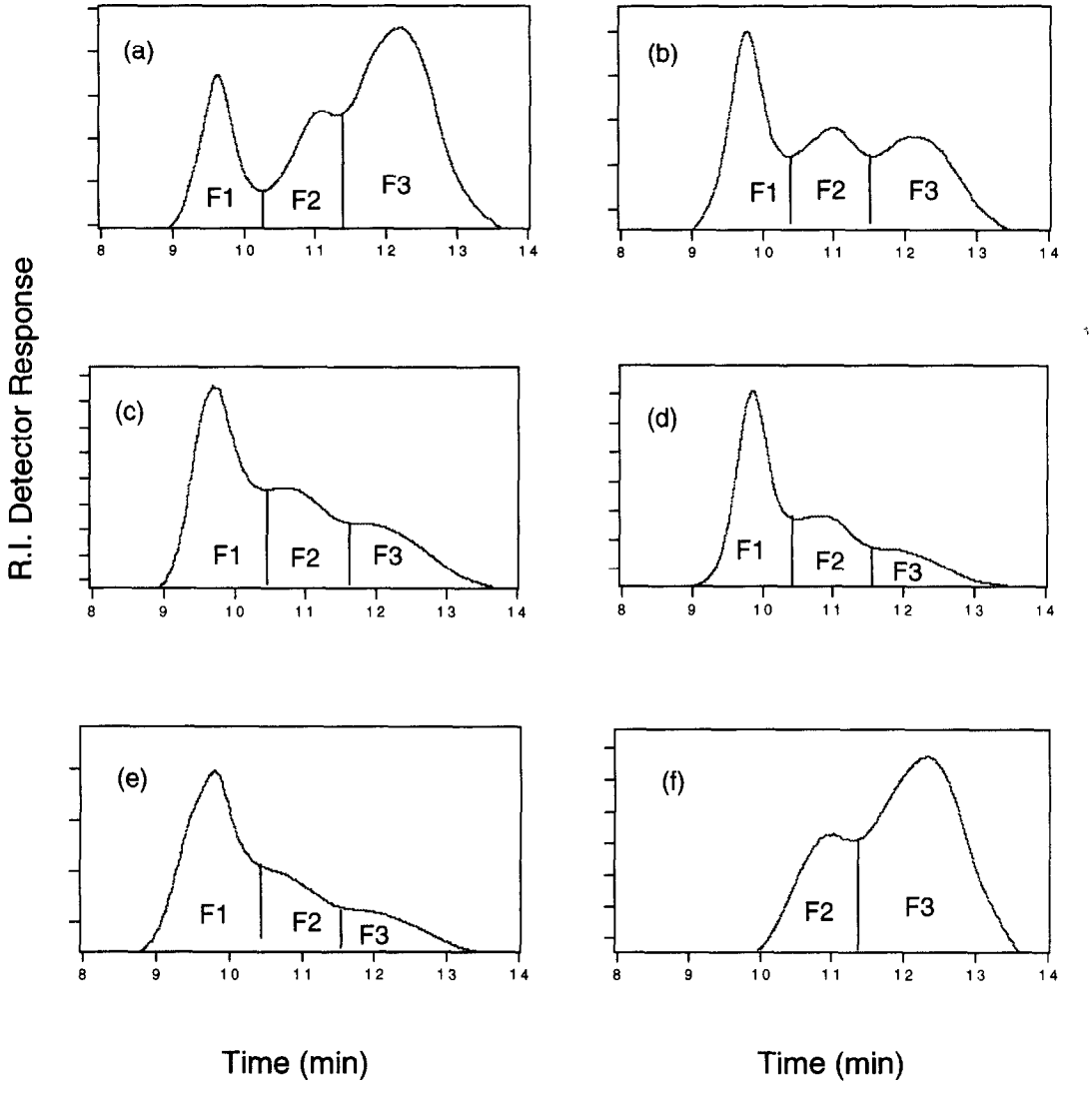

Fig. 8. HPSEC elution profiles of debranched maize starches on the column of Jordi Gel 1000A: (a) MSA, (b) MSB, (c) MSC, (d) MSD, (e) MSE, (f) WMS.

designated long chain and short chain, respectively. The difference among these elution profiles is in their relative ratio of these fractions. MSA has the best separation of fraction 1 and 2, showing little presence of an intermediate fraction. The elution profile of MSB also shows three fractions with clear divisions, but no baseline separation could be obtained, indicating the presence of a large amount of intermediate materials. All high amylose debranched maize starches show similar shapes in elution profiles, suggesting similarity in their structures. The large overlap between F1 and F2 indicates the presence of a large amount of intermediate materials, which reflects the main feature of the fine structure of high amylose starch.

The amylose contents of six maize starches obtained by four methods are summarized in Table 3. The amylose content of the starch determined by method 1 was calculated from iodine affinity (Takeda *et al.*, 1983), which was determined by standard potentiometric titration (Schoch, 1964); the amount of amylose by method 2 was determined from the blue value at 600 nm, using a calibration curve prepared from samples of known amylose content. The percentage of amylose determined by method 3 and method 4 was calculated from the relative area ratio of the amylose fraction and all the fractions considered, which are separated from starch and debranched starch by HPSEC on PL-gel 10 μm size exclusion columns and a Jordi Gel 1000A column respectively (Chuang and Sydor, 1987).

The amylose content of maize starch calculated from HPSEC results was obviously lower than those measured by titration with iodine or colorimetric methods. These are consistent with the results of other researchers (Bradbury and Bello, 1993). Extended hydrolysis time caused no significant change in amylose content. Isoamylase debranched amylopectin of waxy maize does not show a peak near the void volume on Jordi Gel 1000A fractionation, which confirms the absence of a long-chain linear fraction from amylose. For MSA and MSB, which have low amylose content, similar amylose contents could be obtained by four measurement methods. With increase in amylose content, the values determined by iodine-complex methods are higher than those determined by HPSEC. These overestimated values indicate that the long chains in the amylopectin fraction can contribute to the values obtained by the iodine-binding method. When starch has low amylose content, amylopectin has a normal chain length (see Table 2), and the low iodine-binding capacity of amylopectin has little effect on the accurate estimation of amylose content. With an increase in amylose content, the external chain length of amylopectin increases significantly; thus, its increased iodine-binding capacity caused the overestimated value for amylose content. In contrast, HPSEC separates amylose and debranched amylopectin according to their molecular size. With an increase in amylose content, the molecular size decreases, but the long chain length of amylopectin increases. Therefore, the HPSEC of debranched high amylose maize starch shows greater extent of overlapping (intermediate fraction) between the amylose fraction and the long-chain fraction of amylopectin. In addition, the loss of partly branched amylose possibly causes the decrease of apparent amylose content (Banks and Greenwood, 1975). Thus, the intermediate fraction of high amylose maize starch and the greater long chain length in the corresponding amylopectin are the main reasons for the difference in measurement. The differences between method 1 and method 2 for the amylose content in maize starch may be attibuted to difficulties in accurately determining the starch concentration due to the tendancy for amylose to retrograde in aqueous solution.

CONCLUSION

(1) Amylose content in maize starches has a strong correlation with the molecular size of its amylose, the molecular size of amylose decreasing with the increase in amylose content. There is a linear relationship between amylose content and molecular size which can be characterized by $-\log K_{wav}$ and DP values.

(2) Amylose content has a strong correlation with the average chain length of the corresponding amylopectin, the average chain length for longer chains (subfraction 1), and the weight ratio and mole ratio of long chains to short chains. These

Table 3. Comparison of amylose content of maize starches from six types by different methods

	Waxy maize	MSA	MSB	MSC	MSD	MSE
Method 1[a]	0	28	40	56	65	84
Method 2[b]	0	27	45	58	68	80
Method 3[c]	2	24	46	60	62	65
Method 4[d]	0	26	39	51	58	62

[a] Determined by titration with iodine.
[b] Determined by colorimetric analysis.
[c] Determined by butanol-complex/GPC analysis.
[d] Determined by GPC analysis for debranched maize starches.

parameters all increase with the increase in amylose content. The effect of amylose content on the average chain length of amylopectin is the most prominent (slope is 0.175). This suggests that amylopectin molecular size will increase with amylose content in maize starches. This is a possible reason why high amylose maize starch has unique properties.

(3) There is a difference between the amylose content determined by HPSEC and that determined by iodine binding. The iodine-complexation method gives a higher value for high amylose starch, caused by the large amount of longer chains in amylopectin which can complex with iodine.

Although the relationship between amylose content and molecular size of amylose and the distribution of amylopectin chain length presented in this paper was obtained from analytical results of limited samples, the amylose content in these samples has a big range (0–84%), and the results have a very high linear correlation coefficient. So these results should be considered strong evidence to support the above conclusions.

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