Available online at www.sciencedirect.com
SCIENCE @ DIRECT®

Carbohydrate Polymers 55 (2004) 265–272

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Structural changes of corn starches by heating and stirring in DMSO measured by SEC-MALLS-RI system

Jung-Ah Han, Seung-Taik Lim*

Graduate School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, South Korea

Received 9 May 2003; revised 29 July 2003; accepted 29 September 2003

Abstract

The molecular structure of corn starches of different amylose contents (waxy, normal, and high amylose) was analyzed in 90% dimethyl sulfoxide (DMSO) solution by a medium-pressure size-exclusion chromatography connected to refractive index (RI) and multi-angle laser light scattering (MALLS) detectors. The starches were purified as amorphous powders using DMSO and ethanol, and then dissolved in aqueous DMSO (90%) using different boiling and stirring times. For starch dissolution, boiling was required, and subsequent stirring at room temperature increased the starch solubility, measured by filtering (5.0 μm pore size). After 1 h boiling followed by 24 h stirring at room temperature, waxy and normal corn starches were completely soluble in the DMSO solution. For high amylose corn starch, however, 8 h stirring after 1 h boiling yielded complete dissolution, indicating that amylopectin is more difficult to dissolve than amylose. However, excessive boiling (2 h or longer), autoclaving (121 °C, 15 min), or even excessive mechanical stirring caused starch chain degradation. For minimum chain degradation with good solubility, three corn starches were dissolved by stirring for 8 h after boiling for 1 h. Under the condition, waxy corn starch showed a weight-average molecular weight (M_w) of 254×10^6 g/mol, and the M_w's of amylopectin and amylose in normal corn starch were 243×10^6 and 3.13×10^6 g/mol, respectively. However, some amylose chains in normal corn starch tended to aggregate by stirring. Those in high amylose corn starch were 197×10^6 and 1.49×10^6 g/mol, respectively. Therefore, as the amylose level increased in corn starch, the M_w of the amylopectin decreased. The amylose contents in normal and high amylose corn starches, measured by chromatography under the selected dissolution condition, were 28.2 and 74.5%, respectively.

Keywords: Starch; Dimethyl sulfoxide; Boiling; Stirring; Degradation

---

1. Introduction

The use of starch or starch-containing crops in foods or non-food products commonly requires dissolution in aqueous media. To dissolve starch, heating and/or stirring are usually done, however, starch chains solubilized in neutral aqueous solution have limited stability, and readily undergo physico-chemical changes in the solution. For the same reason, the structural analysis of starch is difficult in aqueous media. Moreover, high and inaccurate molar masses are often measured due to incomplete dissolution or chain aggregation (Yokoyama, Renner-Nantz, & Shoemaker, 1998).

Alkaline solutions are often used to obtain complete dissolution, but alkali may induce undesirable side reactions including depolymerization and oxidation. Another solvent commonly used for starch is dimethyl sulfoxide (DMSO). Everett and Foster (1959) were among the first users of DMSO for starch, and they reported that starch solubilization was achieved in this solvent without structural degradation. To disperse starch effectively in DMSO, a small amount of water is necessary to prevent the rapid swelling of starch granules or particles, because surface gel layer, formed by the swelling, would hinder DMSO penetrating the granule (Peng & Perlin, 1987).

The DMSO has been used as a mobile phase in size exclusion chromatography (SEC) system (Kobayashi, Schwartz, & Lineback, 1985; Chung & Sydor, 1987; Yokoyama et al., 1998; Radosta, Hagerer, & Vorwerg, 2001; Vorwerg, Radosta, & Leibnitz, 2002). However, pressurized SEC columns, which have been used exclusively, could cause molecular degradation, due to pressure and friction (Barth & Carlin, 1984). In order to minimize

---

* Corresponding author. Tel.: +82-2-3290-3435; fax: +82-2-9275201.
E-mail address: limst@korea.ac.kr (S.-T. Lim).

0144-8617/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2003.09.007

this degradation, medium- or low-pressure SEC is desirable. Dintzis and Tobin (1974) reported that, although DMSO is known to be a better solvent for starch than water, the use of polar column packing is undesirable, because of the possible interactions between column media and starch. Polymer–polymer, polymer–solvent, and polymer-support interactions can also occur when polar organic solvents are used as mobile phase (Omorodian, Hamielec, & Brash, 1981). These interactions could be eliminated by adding minor content of low M_w electrolytes (e.g. sodium nitrate or lithium bromide) to the mobile phase (Chuang & Sydor, 1987; Meehan, 1995; Yokoyama et al., 1998). To determine M_w with reduced possibility of mechanical degradation and other undesirable interactions, some researchers have employed alternative fractionation techniques including sedimentation field flow fractionation (FFF) (White, 1997; Roger, Baud, & Colonna, 2001; van Bruijnsvoort, Wahlund, Nilsson, & Kok, 2001) and density-gradient ultracentrifugation (Yoon & Lim, 2003).

Millard, Dintzis, Willett, and Klavons (1997) suggested that shear, time, and temperature influenced the physical state of starch dispersed in a solution. However, there is no standard procedure for starch disposal for structural analysis, so various methods have been used to dissolve starch. For example, Kobayashi et al. (1985) dispersed corn and wheat starches in DMSO and heated them in a boiling water-bath for 5 min. Glennie (1987) solubilized sorghum starch in 90% DMSO by heating in a boiling water-bath for 1 h. Chung and Sydor (1987) heated potato starch in pure DMSO at 80 °C for 30 min prior to subjecting to a SEC system. Millard, Wolf, Dintzis, and Willett (1999) used a steam jet to dissolve waxy corn starch in DMSO, and Radosta et al. (2001) swelled potato starch at 80 °C for 24 h, and then heated it at 120 °C for 5 h with stirring.

In the present study, corn starches with different amylose contents were subjected to different heating and stirring conditions to dissolve in 90% DMSO, and the effect of the dissolution conditions on the molecular structure of the starches, using a medium-pressure SEC connected to a multi-angle laser light scattering (MALLS) and refractive index (RI) detectors, was investigated. The molecular characteristics of the three starches tested under a selected dissolution condition for good solubility with minimum degradation were compared.

2. Materials and methods

2.1. Materials

Waxy and normal corn starches were provided by Samyang Genex Co. (Seoul, Korea), and high amylose corn starch was obtained from Cerestar USA, Inc. (Hammond, IN, USA). Starches were purified by using DMSO and ethanol. One gram of starch was dispersed in 90% DMSO (100 ml) with heating in a boiling water-bath for 1 h, and then magnetic-stirred at room temperature for 24 h. The purified starch was precipitated and washed by adding ethanol (300 ml), and then vacuum-dried at 30 °C overnight.

2.2. Starch dissolution for SEC

The dry and pure starch (10 mg, dry solids), in a glass vial, was wetted with ethanol (20 μl), and then 90% DMSO (5 ml) was added. The sample vial was capped, and then boiled for 1 h in a boiling water-bath while magnetic stirring. The starch solution was then mildly stirred (at 155 rpm) up to 24 using a magnetic stirrer at room temperature, and at every 4 h, the SEC analysis was performed. For comparison, starch sample solution, boiled for 2 h or autoclaved (121 °C) for 15 min, was also examined.

2.3. Solubility

The starch sample solutions were filtered using a membrane filter (PTFE 5.0 μm, Millipore Corporation, Bedford, MA), and the starch concentrations before and after filtration were determined by the phenol–sulfuric acid colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Solubility was calculated under the assumption that the soluble starch solely passed the membrane filter.

2.4. SEC-MALLS-RI system

The mobile phase used for SEC was 90% DMSO (HPLC grade) containing LiBr (50 mM), that had been filtered through 0.2 μm Nylon 66 membrane filter (Supelco, PAA). The SEC column (2.6 × 70 cm) contained Toyopearl HW 65 F resins (Tosoh Corp. Tokyo, Japan) of particle and pore sizes, 30–60 μm and 1000 Å, respectively. The system consisted of a pump (P2000, Spectra System, San Jose, CA), an injector valve with a 1 ml loop (model 7072, Rheodyne, Cotati, CA), a SEC column, a MALLS detector (632.8 nm, DAWN DSP-F, Wyatt Technology, Santa Barbara, CA), and an RI detector (Optilab DSP, Wyatt Technology, Santa Barbara, CA). The flow rate was 0.8 ml/min, and the pump pressure 40 psi. The starch solutions, treated under different heating and stirring conditions and filtered through the 5.0 μm PTFE membrane filter, were injected into the SEC column. The specific RI increment value (dn/dc) of the corn starches in DMSO was found to be 0.074 ml/g (Millard et al., 1997).

3. Results and discussion

3.1. Molecular characteristics of normal corn starch

Table 1 shows the structural analysis data for normal corn starch, which was subjected to different dissolution

Table 1
Molecular characteristics of normal corn starch treated under different dissolution conditions

Treatments	M_w ($\times 10^6$ g/mol)		R_g (nm)		Amylose content (%)	Solubility (%)
	Amylopectin	Amylose	Amylopectin	Amylose		
S-24	155 ± 1.2	2.80 ± 2.3	233 ± 0.3	132 ± 1.8	52.1	95.1 ± 0.5
B-1	124 ± 0.6	1.45 ± 0.7	231 ± 0.2	157 ± 0.3	40.1	92.6 ± 0.5
B-1, S-8	243 ± 0.8	3.13 ± 0.8	247 ± 0.2	164 ± 0.2	28.2	98.4 ± 0.8
B-1, S-24	150 ± 1.1	0.89 ± 1.5	239 ± 0.3	216 ± 0.4	48.1	100 ± 1.2
B-2	175 ± 0.9	0.48 ± 1.0	236 ± 0.3	131 ± 0.4	67.7	100 ± 0.6
A-0.25	216 ± 1.0	2.20 ± 0.6	228 ± 0.2	152 ± 0.3	52.6	100 ± 0.8

S, B, and A indicate stirring, boiling, and autoclaving, respectively, and the numbers indicate treatment period (h).

conditions in 90% DMSO. When the starch solution was magnetically stirred for 24 h at room temperature without boiling (S-24), the starch solubility, as determined by filtration, was 95.1%, and the M_w values of amylopectin and amylose were 155×10^6 and 2.80×10^6 g/mol, respectively. The radius of gyration (R_g) was 233 and 132 nm, respectively, for amylopectin and amylose. The starch sample boiled for 1 h (B-1) exhibited the solubility and M_w slightly lower than the values for the stirred sample (S-24). However, when the starch sample was stirred for 8 h at room temperature after boiled for 1 h (B-1, S-8), the starch solubility and M_w increased markedly (Table 1). It indicates that the combined treatments (heating and low temperature stirring) were effective in starch dissolution. As the stirring time was increased to 24 h, complete dissolution was achieved, but the M'_ws of amylopectin and amylose were lower than the values determined after 8 h stirring. The M_w decrease proved chain degradation could occur even by the mechanical stirring. The amylose content was 28.2%, when stirred for 8 h after boiled for 1 h, in agreement with the literature value (~27%). But it became substantially higher after 24 h stirring, revealing that the chain degradation might occur in the amylopectin fraction, resulting in the elution of the degraded amylopectin chains with amylose chains. Amylose was also degraded, as shown in Table 1, showing the M'_ws decrease accordingly with the extensive stirring (B-1, S-24). The R_g value for amylopectin did not change significantly in the range of the heating and stirring applied. But it was noteworthy that the R_g value for amylose continued increasing with stirring, despite of M_w decrease. This trend proved that the three dimensional structure of amylose chains continually changed by the mechanical stirring, likely towards more extended structure, based on the R_g data.

To dissolve normal corn starch in DMSO completely, sufficient mechanical stirring after boiling (1 h) was required, but the results showed that excessive stirring could induce starch chain degradation. From the analysis at 4 h intervals during stirring, stirring for 8 h after boiling 1 h adequately dissolved the purified normal corn starch powder in 90% DMSO. When the starch solution was boiled for 2 h (B-2), the M_w (R_g) values of amylopectin and amylose were 175×10^6 g/mol (236 nm) and 0.48×10^6 g/mol (131 nm), respectively. These values were similar to those determined after 16 h stirring after 1 h boiling (data not shown). By autoclaving the starch solution at 121 °C for 15 min (A-0.25), complete dissolution was obtained, but the M'_ws of amylopectin and amylose were slightly lower than those obtained by boiling for 1 h and stirring for 8 h. But the amylose content was substantially higher (52.6%), indicating the presence of thermal degradation of amylopectin.

Fig. 1 shows the molecular weight distribution of normal corn starch samples treated for different boiling and stirring periods. It clearly shows that the chain structure of normal corn starch is susceptible to boiling and stirring. The amylose fraction displayed more significant M_w change than did the amylopectin fraction. When the stirring was done for 24 h (B-1, S-24), the amylose peak spreaded towards the lower M_w region, generating a separate peak for the low M_w amylose fragments (Fig. 1). When the starch was boiled for 2 h (B-2), chain degradation was most significant, as shown in the chromatogram. A substantial amount of amylose fraction relocated in the low M_w region below 1×10^5 g/mol, and the amylopectin peak became minimized.

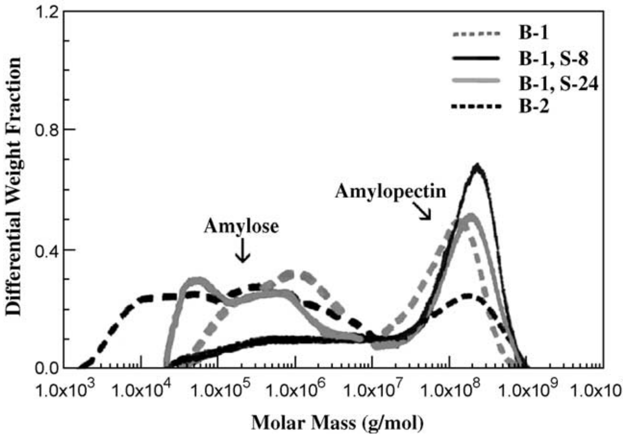

Fig. 1. Differential molar mass distribution for normal corn starch treated under different dissolution conditions (S, and B indicate stirring, boiling, respectively, and the numbers indicate treatment hour).

3.2. Molecular characteristics of waxy corn starch

Table 2 shows the structural analysis results for waxy corn starch, which was dissolved in 90% DMSO under different conditions. When the starch solution was stirred for 24 h at room temperature without heating (S-24), solubility, as measured by filtering recovery, was 92.3%. Boiling for 1 h (B-1) made the starch slightly less soluble (90.3%), but stirring for 24 h after 1 h boiling (B-1, S-24) made the starch completely soluble. M_w and R_g were highest by stirring for 8 h (B-1, S-8), although the solubility was slightly less than 100%. The highest M_w and R_g by 8 h stirring had been observed also with normal corn starch (Table 1). Comparing with the B-1 sample, the solubility increase (~7%) by additional stirring (8 h) might be from the dissolution of high M_w amylopectin that remained insoluble just by 1 h boiling. Hanselmann, Ehart, and Widmer (1995) postulated that incomplete solubilization of starch polymer could lead to an under-estimation of the M_w. Vorwerg et al. (2002) also reported that a small portion of undissolved amylopectin might be retained in the membrane filter prior to the SEC analysis, and it could result in lower M_w measurement. However, as found with normal corn starch, the M_w decrease was observed by excessive stirring, longer than 8 h, which indicates the presence of starch degradation. When the starch solution was boiled for 2 h (B-2) or autoclaved 121 °C for 15 min (A-0.25), its solubility was lower than that after boiling for 1 h and stirring for 24 h (B-1, S-24). It suggests that the mild stirring followed by boiling is very effective in dissolving waxy corn starch in DMSO.

Fig. 2 shows the molecular distribution of waxy corn starch samples treated differently. With an ordinary SEC column, the absence of amylose in waxy corn starch allows the amylopectin to elute as a narrow peak at the void volume of the column, because the exclusion M_w limit of column is usually lower than the M_w of amylopectin. However, the molar mass distribution, transformed based on absolute M'_ws values calculated by light scattering intensity measurement, revealed that there were significant M_w changes induced by the treatments for dissolution. Boiling for 1 h and then stirring for 8 h at room temperature (B-1, S-8) gave the elution peak at the highest M_w region among the tested samples (Fig. 2), in accordance to the data with normal corn starch. Boiling for 1 h and stirring for 8 h was thus an adequate treatment for the dissolution of both normal and waxy corn starches.

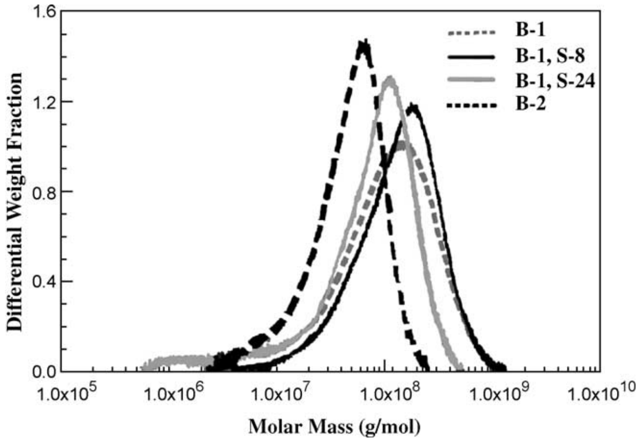

Fig. 2. Differential molar mass distribution for waxy corn starch treated under different dissolution conditions (S, and B indicate stirring, boiling, respectively, and the numbers indicate treatment hour).

Table 2
Molecular characteristics of waxy corn starch treated under different dissolution conditions

Treatment	M_w ($\times 10^6$ g/mol)	R_g (nm)	Solubility (%)
S-24	188 ± 2.1	236 ± 4.1	92.3 ± 1.6
B-1	176 ± 1.6	226 ± 0.3	90.3 ± 1.2
B-1, S-8	254 ± 1.4	241 ± 0.3	97.5 ± 0.8
B-1, S-24	171 ± 1.0	238 ± 0.3	100 ± 0.5
B-2	182 ± 1.2	235 ± 0.2	97.6 ± 0.5
A-0.25	95.1 ± 2.1	213 ± 0.3	99.8 ± 1.3

S, B, and A indicate stirring, boiling, and autoclaving, respectively, and the numbers indicate treatment period (h).

3.3. Molecular characteristics of high amylose corn starch

Table 3 shows the molecular structural data for high amylose corn starch dissolved in 90% DMSO under different conditions. When the starch solution was stirred for 24 h at room temperature without boiling (S-24), the M'_ws of the amylopectin and amylose were 171×10^6 and 1.40×10^6 g/mol, respectively, and the solubility was near 100% (98.2%). When boiled for 1 h (B-1), the solubility and the M'_ws of the amylopectin and amylose were almost identical to the values of S-24 sample. Amylose content as determined by RI chromatogram was also similar (72.3 and 72.5%). When the solution was stirred for 8 h after 1 h boiling (B-1, S-8), complete dissolution was achieved, and the M'_ws of amylopectin and amylose slightly increased (197×10^6 and 1.49×10^6 g/mol, respectively). After 24 h stirring, however, the M_w of amylopectin decreased slightly to 181×10^6, indicating the presence of shear-induced degradation. It was noteworthy that the amylose content was little changed although molecular degradation occurred.

In 90% DMSO, the high amylose starch exhibited 100% solubility after 8 h stirring after 1 h boiling, whereas the waxy and normal corn starches required 24 h of stirring for complete dissolution. It indicates that the starch containing more amylopectin is more difficult to dissolve. Boiling for 2 h (B-2) or autoclaving for 15 min (A-0.25) induced M_w decrease, as did normal and waxy starches. Comparing the molar mass distributions of waxy or normal corn starch, the change of M_w of high amylose starch caused by heating and stirring was not as significant as those found with waxy and normal starches, except B-2 sample (Fig. 3). This result suggests that high amylose corn starch, which has a relatively lower M_w than normal or waxy starch, was less

Table 3
Molecular characteristics of high amylose corn starch treated under different dissolution conditions

Treatment	M_w (× 10^6 g/mol)		R_g (nm)		Amylose content (%)	Solubility (%)
	Amylopectin	Amylose	Amylopectin	Amylose		
S-24	171 ± 2.0	1.40 ± 1.8	236 ± 1.2	82 ± 0.5	72.3	98.2 ± 0.8
B-1	171 ± 1.5	1.43 ± 1.0	245 ± 0.3	80 ± 0.6	72.5	98.3 ± 0.6
B-1, S-8	197 ± 2.2	1.49 ± 0.6	214 ± 0.5	83 ± 0.3	74.5	100 ± 0.2
B-1, S-24	181 ± 1.9	1.51 ± 1.5	237 ± 0.4	84 ± 0.3	72.3	100 ± 0.5
B-2	169 ± 0.7	1.45 ± 0.6	233 ± 0.3	114 ± 0.3	77.4	98.5 ± 0.3
A-0.25	123 ± 2.3	1.38 ± 0.8	239 ± 0.5	90 ± 0.2	80.8	100 ± 0.6

S, B, and A indicate stirring, boiling, and autoclaving, respectively, and the numbers indicate treatment period (h).

affected by the physical treatments used for dissolution. However, as shown in Fig. 3, extensive boiling (B-2) resulted in significant chain degradation both in amylose and amylopectin chains.

Autoclaving has often been used to improve starch solubility. Aberle, Burchard, Vorwerg, and Radosta (1994) reported that autoclaving of an aqueous starch solution at 135–155 °C was effective, though the temperature depended on starch origin. Hanselmann et al. (1995) used a higher temperature under pressure (175 °C) for the dissolution of waxy corn starch. You and Lim (2000) reported that autoclaving (121 °C, 20 min) provided a better recovery and higher M_w from an aqueous high performance size exclusion chromatography (HPSEC) for wheat amylopectin dissolved in water, than atmospheric boiling. Vorwerg et al. (2002) used heating under pressure (150 °C, 20 min) to obtain a molecularly dispersed aqueous solution for pea starch. Millard et al. (1997) autoclaved (130 °C, 45 min) a dispersion of waxy corn starch in DMSO, and obtained M_w (R_g) of 586×10^6 g/mol (376 nm) by micro-batch mode analysis. Radosta et al. (2001) autoclaved (121 °C, 5 h) waxy potato starch in 90% DMSO, and reported that the M_w's (R_g's) from batch and HPSEC measurements were 37×10^6 g/mol (130 nm), and 44×10^6 g/mol (175 nm), respectively. But for the purified starch powder used in the present study, autoclaving, even at the lower temperature (121 °C) than those in the literatures, rendered substantial changes in the molecular structure of the starch, depending sensitively on the autoclaving period. Thus, to achieve complete dissolution without thermal degradation of starch may be difficult with the autoclaving treatment.

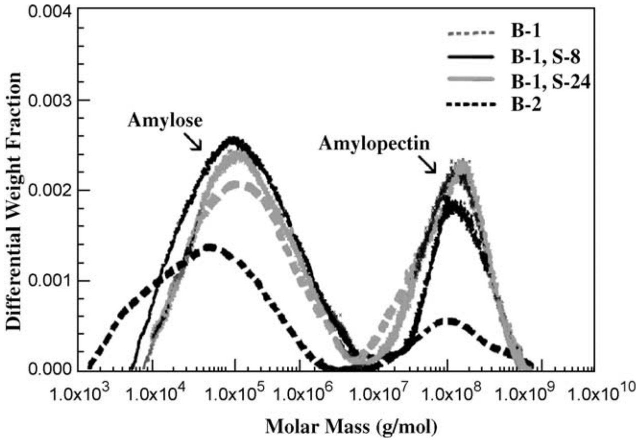

Fig. 3. Differential molar mass distribution for high amylose corn starch treated under different dissolution conditions (S, and B indicate stirring, boiling, respectively, and the numbers indicate treatment hour).

In our preliminary experiments, M_w and solubility were affected also by the membrane filters used prior to SEC analysis. When a nylon filter (5.0 μm, Whatman, UK) was used, autoclaving (121 °C) for 60 min was required to obtain a good solubility (92.8%) for waxy corn starch. However, when a PTFE filter of the same pore size (Millipore, Bedford, MA) was used, starch recovery was complete under the same dissolution condition, but the M_w measured was substantially lower. Thus it was hypothesized that some starch molecules, especially large amylopectins, were retained by the nylon filter, possibly by interactions with the filter material. So proper selection of the filter is important to obtain accurate structural data from SEC analysis.

3.4. Comparison of three corn starches under a selected dissolution condition

Table 4 shows the comparison in the structural characteristics of three corn starches containing different amylose contents, treated under a selected dissolution condition, i.e. boiling for 1 h and stirring for 8 h. Waxy corn starch showed the highest M_w for amylopectin, and high amylose corn starch showed the lowest M_w for amylopectin. It was noteworthy that the R_g value of amylopectin was not very different among the three starches tested. However, the amylose in normal corn starch had an R_g value that was double of that of the amylose in high amylose corn starch. The specific volume for gyration (SV_g), calculated assuming the chains have a spherical gyration (You & Lim, 2000), was 0.14 for waxy corn amylopectin, but those of normal and high amylose corn amylopectins were slightly larger (0.16 and 0.23, respectively). This suggests that the amylopectin molecules in waxy, normal and high amylose corn starches are somewhat different in molecular structure. High amylose corn

Table 4
Comparison of the structural characteristics of three corn starches in the selected condition

Starches	Amylopectin				Amylose				Solubility (%)
	M_w ($\times 10^6$)	R_g (nm)	SV_g	M_w/M_n	M_w ($\times 10^6$)	R_g (nm)	SV_g	M_w/M_n	
Waxy corn	254 ± 1.4	241 ± 0.3	0.14	1.3	–	–	–	–	97.5 ± 0.8
Normal corn	243 ± 0.8	247 ± 0.2	0.16	1.4	3.13 ± 0.8	164 ± 0.2	3.55	2.1	98.4 ± 0.8
High amylose corn	197 ± 2.2	214 ± 0.3	0.23	1.7	1.49 ± 0.6	83 ± 0.3	0.97	3.8	100 ± 0.2

amylopectin, which has the largest SV_g value, has the least compact structure, possibly due to longer chains and fewer branches than normal or waxy corn amylopectin. Han, BeMiller, Hamaker, and Lim (2003) compared the chain profiles of debranched waxy, normal and high amylose corn starches, and reported that the amylopectin in high amylose corn starch had the long B chains with the largest CL_w, and the short A chains with the least portion.

Under the selected dissolution condition, the M_w and R_g of the amylose in normal corn starch were greater than those of the amylose in high amylose corn starch. Also, there was a significant difference in the SV_g value: 3.55 for normal corn amylose and 0.97 for high amylose corn amylose. It indicates that normal corn amylose has a significantly more extended structure than high amylose corn amylose in DMSO. Han et al. (2003) reported that the long amylose chains from debranched normal corn starch were 20.3%, whereas those from debranched high amylose corn starch (Hylon VII) were 35.9%. The differences between these values and the amylose contents in both starches (approximately 27 and 70%, respectively) are the short amylose chains that eluted with amylopectin chains from SEC chromatography, after debranching. The greater difference for the amylose in high amylose starch (70 vs. 35.9%) indicates that high amylose starch contains shorter amylose chains than does normal starch (20.3 vs. 27%). Takeda, Takeda, and Hizukuri (1989) also reported that amylomaize (high amylose corn) amylose is comprised of branched molecules with shorter inner chains than normal maize amylose. The short inner chains, therefore, allow the amylose in high amylose corn starch to form more compact structure, as proved by the low SV_g value (0.97) measured in this experiment.

Three-dimensional conformation of amylose in DMSO has been controversial for many years. Banks and Greenwood (1968) reported that the basic skeletal structure of amylose molecules must be the same in neutral, alkaline, or DMSO solution, and the only molecular configuration of amylose was a random coil in the solutions, without a helical character, based on intrinsic viscosity $[\eta]$ and M_w. Nakanish, Norisuye, and Teramoto (1993) measured amylose conformation in DMSO, based on M_w, second virial coefficient (A_2), radius of gyration, and intrinsic viscosity $[\eta]$, and reported that the amylose chains below $M_w \sim 10^4$ had a helical worm like structure in DMSO, but that the overall chain conformation at $M_w > 10^5$ was like random coil, expanded by the excluded volume effect. Radosta et al. (2001) suggested that amylose behaved like a flexible chain in a thermodynamically good solvent, like DMSO in this case. On the other hand, Jacques, Sundararajan, Taylor, and Marchessault (1976) found intramolecular hydrogen bonding in NMR study which was necessary to form a helical structure. Cheetham and Tao (1998) also reported that adding water to pure DMSO reduced the interaction between amylose and DMSO, leading to a conformational transition of amylose from tight helix to loose helix or disordered coil.

In the present study, the SV_g of normal corn amylose dissolved in DMSO (3.55) was substantially greater than that (1.0) measured in an aqueous solvent (You & Lim, 2000). Although the present data were not sufficient to suggest exact conformation of amylose, the difference in SV_g confirmed the fact that amylose has a more extended conformation in DMSO than in water. Moreover, the SV_g of starch chains, among the different starches tested, provides useful approximation of their chain structures, in chain length and degree of branching.

The polydispersity (M_w/M_n) of amylopectin was positively related with amylose content (Table 4). The values for amylopectins, ranged from 1.3 to 1.7, depended on the amylose content, which linearly raised the amylopectin polydispersity. Polydispersity of amylose is usually greater than that of amylopectin, because amylose chains distribute in broader molecular weight range than do amylopectin chains. Amylose presence may alter the structure formation of amylopectin during their synthetic procedures, or some amylose chains, in proximity to amylopectin structure, reside with amylopectin as contaminants.

3.5. Possible structural changes by stirring

The analytical data revealed that starch chains, both amylose and amylopectin, are highly susceptible to mild and low temperature stirring. In other words, the stirring increases starch solubility, but changes M_w, either raising or reducing depending on stirring periods. For normal corn starch, when the boiled starch solution was treated at different stirring periods (up to 24 h), the maximum M_w's for both amylose and amylopectin were obtained at 8 h stirring, but the least amylose content was also measured (Table 1). The solubility increase by the additional 8 h stirring, from 92.6 to 98.4%, was assumed from the excess dissolution of exceptionally high M_w amylopectin chains, which thus resulted in M_w increase for amylopectin. But the increment (5.8%) was significantly too low to attribute the amylose content difference (28.2 and 40.1%). If the amylose content (28.2%) for the B-1, S-8 sample is correct, the high amylose content in B-1 sample (40.1%) may suggest that a part of amylopectin eluted with amylose in SEC analysis. But amylopectin elution in the amylose fraction should have raised the M_w of amylose, which was not true in this data (B-1, in Table 1). Therefore, the abrupt change in amylose content (40.1–28.2%) with the concurrent M_w increases for both amylose and amylopectin by stirring for 8 h can be explained only by the molecular entanglements. Due to the mechanical shearing, the linear amylose chains tended to associate, and the associates eluted in the amylopectin fraction. And the chain association resulted in the M_w increases for both amylose and amylopectin fractions. The starch sample stirred for 24 h displayed the reduced M_w's but increased amylose content. In this case, chain degradation induced these changes. And the same result was observed with waxy corn starch samples (Table 2), displaying the maximum M_w for B-1, S-8 sample. For high amylose corn starch, the M_w of amylopectin was highest for B-1, S-8 sample, too. But the effect of stirring was much less than the case for normal or waxy corn starch. As indicated with the R_g value, high amylose corn starch contained the amylose of shorter and more branched chains, than did normal corn starch, as reported by Takeda et al. (1989) and Han et al. (2003). The short and branched chains have fewer tendencies to aggregate and thus the stirring effect became less. Starch chain aggregation by mechanical stirring was obvious in aqueous media (Han et al., 2003). Although starch in DMSO is more stable in conformation change, the present data suggest the possibility of the chain aggregation. To confirm amylose aggregation effect, additional study should be followed.

4. Conclusions

The use of medium-pressure size exclusion column and 90% DMSO as starch solvent and chromatography eluent might reduce starch degradation or aggregation induced by thermal and mechanical treatment for dissolution, and by pressure and friction in column, which often cause inaccurate results in structural analysis when high pressure column and aqueous media are used. DMSO was found to dissolve the starch completely with proper combination of boiling and mild stirring. Boiling for 1 h and subsequent stirring at room temperature for 8 h was needed to properly dissolve the amorphous powders of waxy, normal or high amylose corn starch in 90% DMSO for SEC analysis. But, excess boiling or stirring causes molecular degradation, and the structural changes occur more significantly in amylose fraction than in amylopectin fraction. Amylopectin was more difficult to dissolve than amylose, and thus a waxy starch required more stirring for complete dissolution than a high amylose corn starch. For accurate molecular characterization, careful control of starch dissolution condition is required.

References

Aberle, T., Burchard, W., Vorwerg, W., & Radosta, S. (1994). Conformational contributions of amylose and amylopectin to the structural properties of starches from various sources. *Starch/Starke*, *46*, 329–335.

Banks, W., & Greenwood, C. T. (1968). The hydrodynamic behavior of native amylose in good solvents. *Carbohydrate Research*, *7*, 414–420.

Barth, H. G., & Carlin, F. J. (1984). A review of polymer shear degradation in size-exclusion chromatography. *Journal of Liquid Chromatography*, *7*, 1717–1738.

Cheetham, N. W. H., & Tao, L. (1998). Amylose conformational transitions in binary DMSO/water mixtures. *Carbohydrate Polymers*, *35*, 287–295.

Chuang, J.-Y., & Sydor, R. J. (1987). High performance size exclusion chromatography of starch with dimethyl sulfoxide as the mobile phase. *Journal of Applied Polymer Science*, *34*, 1739–1748.

Dintzis, F. R., & Tobin, R. J. (1974). Molecular-sieve chromatography of amylose and dextran over porous glass. *Journal of Chromatography A*, *88*, 77–85.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Everett, W. W., & Foster, J. F. (1959). The subfractionation of amylose and characterization of the subfractions by light scattering. *Journal of the American Chemical Society*, *81*, 3459–3464.

Glennie, C. W. (1987). Physico-chemical properties of sorghum starch thermally treated by different methods. *Starch/Starke*, *39*, 273–276.

Han, J.-A., BeMiller, J. N., Hamaker, B., & Lim, S.-T. (2003). Structural changes of debranched corn starch by aqueous heating and stirring. *Cereal Chemistry*, *80*, 323–328.

Hanselmann, R., Ehart, M., & Widmer, H. M. (1995). Sedimentation field flow fractionation combined with multi-angle laser light scattering applied for characterization of starch polymers. *Starch/Starke*, *46*, 345–349.

Jacques, M., Sundararajan, P. R., St, Taylor, K. J., & Marchessault, R. H. (1976). Nucleo magnetic resonance and conformational studies and model compounds in dimethyl sulfoxide solution. *Journal of the American Chemical Society*, *98*, 4386–4391.

Kobayashi, S., Schwartz, S. J., & Lineback, D. R. (1985). Rapid analysis of starch, amylose and amylopectin by high-performance size-exclusion chromatography. *Journal of Chromatography*, *319*, 205–214.

Meehan, E. (1995). Semirigid polymer gels for size exclusion chromatography. In Wu Chi-san (Ed.), *Handbook of size exclusion chromatography* (pp. 25–44). New York: Marcel Dekker.

Millard, M. M., Dintzis, F. R., Willett, J. L., & Klavons, J. A. (1997). Light-scattering M_w's and intrinsic viscosities of processed waxy maize starches in 90% dimethyl sulfoxide and H_2O. *Cereal Chemistry*, *74*, 687–691.

Millard, M. M., Wolf, W. J., Dintzis, F. R., & Willett, J. L. (1999). The hydrodynamic characterization of waxy maize amylopectin in 90% dimethyl sulfoxide-water by analytical ultracentrifugation, dynamic and static light scattering. *Carbohydrate Polymers*, *39*, 315–320.

Nakanishi, Y., Norisuye, T., & Teramoto, A. (1993). Conformation of amylose in dimethyl solfoxide. *Macromolecules*, *26*, 4220–4225.

Omorodian, S. N., Hamielec, E., & Brash, J. L. (1981). Optimization of peak separation and broadening in aqueous gel permeation chromatography (GPC)-dextrans. *Journal of Liquid Chromatography*, *4*, 41–50.

Peng, O.-J., & Perlin, A. S. (1987). Observations on NMR spectra of starches in dimethyl sulfoxide. Iodine-complexing, and solvation in water-dimethyl sulfoxide. *Carbohydrate Research*, *160*, 57–72.

Radosta, S., Hagerer, M., & Vorwerg, W. (2001). Molecular characteristics of amylose and starch in dimethyl sulfoxide. *Biomacromolecules*, *2*, 970–978.

Roger, P., Baud, B., & Colonna, P. (2001). Characterization of starch polysaccharides by field-flow fractionation—multi-angle laser light scattering-differential refractometer index. *Journal of Chromatography A*, *917*, 179–185.

Takeda, C., Takeda, Y., & Hizukuri, S. (1989). Structure of amylomaize amylose. *Cereal Chemistry*, *66*, 22–25.

van Bruijnsvoort, M., Wahlund, K. G., Nilsson, G., & Kok, W. Th. (2001). Retention behavior of amylopectins in asymmetrical flow field-flow fractionation studied by multi-angle light scattering detection. *Journal of Chromatography A*, *925*, 171–182.

Vorwerg, W., Radosta, S., & Leibnitz, E. (2002). Study of a preparative-scale process for the production of amylose. *Carbohydrate Polymers*, *47*, 181–189.

White, R. J. (1997). FFF-MALS A new too for the characterization of polymers and particles. *Polymer International*, *43*, 373–379.

Yokoyama, W., Renner-Nantz, J. J., & Shoemaker, C. F. (1998). Starch molecular mass and size by size-exclusion chromatography in DMSO-LiBr coupled with multiple angle laser light scattering. *Cereal Chemistry*, *75*, 530–535.

Yoon, J.-W., & Lim, S.-T. (2003). Molecular fractionation of starch by density-gradient ultracentrifugation. *Carbohydrate Research*, *338*, 611–617.

You, S., & Lim, S.-T. (2000). Molecular characterization of corn starch using an aqueous HPSEC-MALLS-RI system under various dissolution and analytical conditions. *Cereal Chemistry*, *77*, 303–308.