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Structural changes in corn starches during alkaline dissolution by vortexing

Jung-Ah Han, Seung-Taik Lim*

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

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Abstract

Corn starches of different amylose contents (waxy, normal, and high amylose) were dissolved in a strong alkaline solution (1 M NaOH) by vigorous vortexing at room temperature, and analyzed by a medium-pressure size exclusion chromatography (SEC), using 50 mM NaOH as eluent, connected to refractive index and multi-angle laser light scattering detectors. The vortexing increased the solubility of the corn starches, measured as the recovery from filtration (5.0 μm pore size). After 20 min vortexing, the solubility of waxy corn starch reached 89.4%. For normal and high amylose corn starches, however, vortexing for less than 5 min yielded a solubility of more than 90%. It indicates that amylopectin is more difficult to dissolve in alkaline solution than amylose. The size exclusion chromatographic pattern changed with vortexing time, indicating that excess vortexing caused molecular degradation. In the dissolution condition with minimum degradation and good solubility (15 min vortexing), waxy corn starch exhibited an average molecular weight (M_w) and radius of gyration (R_g) as 185×10^6 g/mol and 214 nm, respectively. The M_w (R_g) values of amylopectin and amylose in normal corn starch, following vortexing for the optimal time of 5 min were 164×10^6 (255 nm) and 3.3×10^6 g/mol (154 nm), respectively. The M_w (R_g) of amylopectin and amylose in high amylose corn starch were slightly smaller than those in normal corn starch: 113×10^6 (175 nm) and 2.7×10^6 g/mol (99 nm), respectively.

Keywords: Corn starch; Alkali; Degradation; Molecular weight

1. Introduction

Starch is a major component in many foods, and often added as a functional ingredient in a variety of industrial products (Fredriksson, Silverio, Andersson, Eliasson, & Aman, 1998). Starch is biosynthesized in a granule form, and is primarily composed of two biopolymers, i.e. amylose and amylopectin. The structural characteristics of the polymers, such as average molecular weight (M_w), degree of branching, and chain length influence their physical characteristics. However, accurate analysis of molecular structure is not easy because of their exceptionally large size and high heterogeneity. For structural characterization, the two polymers are usually separated by using various techniques, including size-exclusion chromatography (SEC) (Bello-Perez, Roger, Baud, & Colonna, 1998; Fishman, Rodriguez, & Chau, 1996; Jackson, Choto-Owen, Waniska, & Rooney, 1988), field-flow fractionation (FFF) (Hanselmann, Ehart, & Widmer, 1995; Roger, Baud, & Colonna, 2001), and capillary electrophoresis (Brewster & Fishman, 1995). Among these, SEC, in which the separation is performed on the basis of hydrodynamic volume, is most widely used. The M_w of the separated starch molecules is often determined based on retention or elution time relative to those of standard compounds of known size. However, hydrodynamic density of the standard compounds is different from the starch molecules, so the calculated M_w may be different from the actual values. In contrast, the multi-angle laser light scattering (MALLS) detector provides absolute value of molecular mass without use of the standard compounds, and gives other structural information such as radius of gyration (R_g), and the second virial coefficient (A_2), that relates to the interaction with solvent.

Starch is susceptible to physical treatment. The molecular degradation is often encountered as an obstacle when a pressurized SEC column is used for structural analysis (Barth & Carlin, 1984). In order to minimize the shear and

* Corresponding author. Tel.: +82-2-3290-3435, fax: +82-2-927-5201.
E-mail address: limst@korea.ac.kr (S.-T. Lim).

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pressure-induced degradation, medium or low-pressure SEC may be desirable.

Water or dilute salt solution is commonly used as starch solvent in SEC-based analysis. However, the limited solubility of starch in neutral water or salt solution makes the analysis difficult. To improve the solubility, Fishman and Hoagland (1994) treated corn starches of different amylose contents with microwave heating, and measured their M_w using SEC and MALLS detector to be $3.2-20 \times 10^6$ g/mol. Aberle, Burchard, Vorwerg, and Radosta (1994) also reported the difficulty in measuring starch structure with conventional physical treatment (e.g. boiling and stirring) in aqueous medium. They dissolved corn starches by autoclaving aqueous starch dispersions, and obtained the M_w of waxy, normal, and high amylose corn starches as 76.9×10^6, 88.0×10^6, and 16.7×10^6 g/mol, respectively.

Moreover, aggregation of starch, especially amylose chains readily occurs in water, which often causes inaccurate measurements in structural and physical analyses (Roger & Colonna, 1992). For SEC analysis, some large aggregates of amylose and undissolved amylopectin clusters remain unfiltered (Chen, Fringant, & Rinaudo, 1997), and thus are excluded in the measurement. Incomplete dissolution of starch was reported even when autoclaved (Aberle et al., 1994; Hanselmann et al., 1995; You & Lim, 2000) or microwave-heated (Bello-Perez et al., 1998; Fishman et al., 1996). Therefore, structural analysis data from the same starch sample could give different results depending on the physical treatment for dissolution.

As alternative solvents for starch, dimethyl sulfoxide (DMSO) (Klavons, Dintzis, & Millard, 1997; Millard, Dintzis, Willett & Klavons, 1997) and strong alkaline solution (Jackson et al., 1988; Roger, Tran, Lesec, & Colonna 1996) are frequently used for the structural analysis using SEC system. Suortti and Pessa (1991) reported that refractive index (RI) detector, together with MALLS detector, showed a better sensitivity in the analysis with alkaline solution than with DMSO. Yu and Rollings (1987) used 0.5 N NaOH to dissolve starch, and reported that the alkaline solution prevented amylose aggregation. Roger et al. (1996) obtained reproducible M_w result with amyloses of various origins by using 0.1 M KOH. Roger and Colonna (1992) showed A_2 for the amylose-KOH solution to be $6 \times 10^{-4}-2 \times 10^{-3}$, indicating that the alkaline solution was a good solvent for amylose. However, structural analyses for starch using the SEC system with alkaline eluent have been rare, because most aqueous SEC columns are unstable to strong alkali (Nagamine & Komae, 1996).

In the present study, the effect of vortexing alkaline starch solution on the molecular structure and solubility of corn starches containing different amylose contents was investigated by using a medium-pressure SEC with an alkaline eluent, connected to MALLS and RI detectors.

2. Materials and methods

2.1. Materials

Three corn starches containing different contents of amylose (waxy, normal, and high amylose) were used. Waxy and normal corn starches were obtained from Samyang Genex Co. (Seoul, Korea) and high amylose corn starch (about 70% amylose) was obtained from Cerestar USA, Inc. (Hammond, IN, USA). The starches (1 g, dry solids) were dispersed in 90% DMSO (100 ml) in a boiling water-bath for 1 h, and then magnetic-stirred at room temperature for 24 h. The starch in the solution was precipitated by adding ethanol (3 × volume of DMSO), and then washed with ethanol (3 times) and acetone (once). The purified starch was dried in a convection oven (30 °C).

2.2. Starch solution preparation

Corn starch (10 mg, dry solids) in a glass vial was wetted with ethanol (20 μl), and then dispersed in 1 M NaOH (500 μl). The sample vial was capped, and then was vortexed at room temperature (IKA Co., Staufen, Germany) at 2500 rpm for different periods (2, 5, 10, and 20 min), and then the starch solution was diluted to 50 mM NaOH with water. The diluted solution was gently stirred (155 rpm) using a magnetic stirrer for 30 min at room temperature prior to application to the medium-pressure size-exclusion column.

2.3. Filtration recovery (solubility)

The concentration of starch in the solution before and after filtration using a porous membrane syringe filter (5.0 μm, Acrodisc syringe filter, Pall Gelman Sciences, Ann Arbor, MI) was determined by the phenol-sulfuric acid colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.4. SEC-MALLS-RI system

The mobile phase used for the SEC was 50 mM NaOH solution (pH 12.8), filtered through 0.1 μm cellulose acetate membrane (Whatman, Kent, UK) and degassed. The SEC column (2.6 × 70 cm) contains Toyopearl HW 75 F resins (TosoHaas, PA, USA) with particle and pore sizes of 30–60 μm, and 1000 Å, respectively. The analytical M_w range was from 100,000 to 10,000,000 (based on dextran). The packing material, a hydrophilic vinyl copolymer was resistant to the alkaline eluent.

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), the SEC column, a MALLS detector (632.8 nm, DAWN DSP-F, Wyatt Technology, Santa Barbara, CA), and a RI detector (Optilab DSP, Wyatt Technology, Santa Barbara, CA). Flow rate was 0.8 ml/min,

and the pump pressure was 38 psi. The starch solution, filtered through the 5.0 μm syringe filter, was injected to the column, and specific RI increment value (dn/dc) of 0.146 ml/g was used for starch (Yu & Rollings, 1987).

3. Results and discussion

Alkali affects structure and physical behavior of starch in solution. The majority of −OH groups in the anhydrous glucose units are ionized at high pH (12.5), so agglomeration of starch chains and paste retrogradation can be retarded (Suortti, Gorenstein, & Roger, 1998). However, molecular degradation is accelerated by alkali (Wang & Zopf, 1989). Thus it is important to find the dissolution condition for minimum degradation, but complete solubilization in alkaline solution. The degree and product composition from the alkaline degradation are influenced by several reaction parameters, including temperature, nature and concentration of the alkali, and presence of oxygen (de Bruijn, Kieboom, & van Bekkum, 1986; Knill & Kennedy, 2003; Lai & Sarkanen, 1969; Nevell, 1985; Yang & Montgomery, 1996). Nagamine and Komae (1996) examined the effect of NaOH concentration on the dissolution of debranched amylopectin chains (dry sample), and reported that the dissolution was incomplete in 100 mM NaOH. Especially the longer chains (B2 chains) were found to be more difficult to dissolve. Therefore, stronger alkali, e.g. 1 or 2 M NaOH (Bennow, Bay-Smith, & Bauer, 2001; Kennedy, Rivera, Lloyd, & Warner 1992; Suortti & Pessa, 1991), or KOH (Roger, Axelos, & Colonna, 2000; Roger & Colonna, 1992) is commonly used for starch dissolution for structural analysis. The strong alkali breaks the intermolecular hydrogen bonds of starch, enhancing water solubility.

In this study, amorphous corn starch powders were dispersed in 1 M NaOH, and vigorous vortexing (2500 rpm) was applied for different periods up to 20 min. The vortexing was done at room temperature to minimize any heat-induced degradation. And then the starch solution was diluted to 50 mM by adding water, to reach the eluent concentration. Additional mild magnetic-stirring (155 rpm, 30 min) was also applied at room temperature before injecting on to the size exclusion column.

To examine any effect of oxygen on the molecular degradation, N_2 purging was applied to the starch solutions during the treatment. However, there was no significant difference in the analytical data (M_w and R_g) for the three corn starches tested (data not shown). This might be because no heat was applied and the treatment time was relatively short.

3.1. Molecular characteristics of normal corn starch

Table 1 shows the solubility and structural data for the normal corn starch sample vortexed at different periods in 1 M NaOH. The vortexing times greatly affected starch solubility and structural data. As the vortexing time increased, the solubility increased, but the M_w of amylopectin and amylose decreased. When the starch solution was vortexed for 2 min, about 20% of the starch remained insoluble, and the amylose content measured from SEC was 40.1%. This result indicates that the insolubles were mostly amylopectin. By vortexing for 5 min, the solubility increased to 92.1%, but the M_w of amylopectin and amylose decreased to 164×10^6, and 3.3×10^6 g/mol, respectively. The solubility increase was achieved, further up to 94.9% by 10 min vortexing, but the M_w decrease was more substantial. This trend indicates amorphous dry starch can be dissolved almost completely by vortexing for 10 min in 1 N NaOH solution at room temperature. But chain degradation in both amylose and amylopectin were evident. It was confirmed by the changes in SEC chromatogram (Fig. 1). With a short vortexing (2 min), the elution peaks of amylopectin and amylose overlapped extensively. But, when vortexing was done for 5 min, the separation between amylopectin and amylose became obvious and the relative size of the amylopectin peak became greater. With 10 min vortexing, however, the amylopectin degradation appeared significant, the elution peak spreading into a broader elution range. The amylose content decreased as the vortexing time increased, but its degradation was not as significant as that of amylopectin (Table 1). The decreased amount of amylose in the chromatogram was unexpected, because amylopectin degradation usually causes an apparent amylose content increase. It was hypothesized that some amylose chains were associated with amylopectin chains, making

Table 1
Average molecular weight (M_w), radius of gyration (R_g) and solubility of normal corn starches dissolved in 1 M NaOH at different vortexing periods

Vortexing (min)	M_w ($\times 10^6$ g/mol)		R_g (nm)		Amylose content (%)	Solubility (%)
	Amylopectin	Amylose	Amylopectin	Amylose		
02	237 ± 5.9	4.5 ± 2.7	259 ± 9.0	152 ± 7.0	40.1	80.3 ± 0.2
05	164 ± 1.0	3.3 ± 1.7	255 ± 0.2	154 ± 0.3	24.6	92.1 ± 0.9
10	126 ± 1.1	2.9 ± 1.1	211 ± 0.2	150 ± 0.7	15.2	94.9 ± 1.2

Values are average of three replicates of each sample.

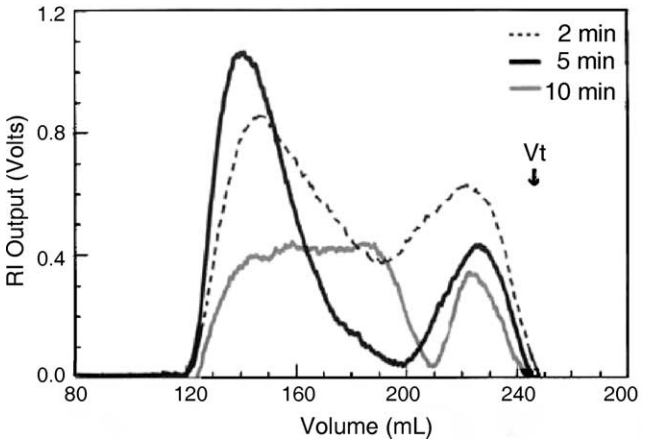

Fig. 1. Refractive index chromatograms of normal corn starches dissolved in 1 M NaOH at different vortexing periods.

the apparent amylose content smaller. Based on this result, of the vortexing times tested, 5 min was the best for the dissolution of dry normal corn starch sample in 1 M NaOH for subsequent SEC analysis.

The radius of gyration of corn amylose in alkaline solution was higher than the value obtained in water or in DMSO (Nakanishi, Norisuye, Teramoto, & Kitamura 1993; Roger et al., 1996). Because, in an aqueous neutral solution, amylose chains were reported to exist as an interrupted helix, but by adding alkali, the hydrogen bonds stabilizing the helix were progressively destroyed and the amylose macromolecules adopted a more random conformation. At pH 12, the helical conformation was supposed to be completely absent (Bank & Greenwood, 1972). In addition, with more alkali addition, a progressive increase in the negative charge on the polymer molecule induced charge repulsion between chains (Foster, 1965).

To elucidate the amylose conformation, a logarithmic plot of M_w against R_g, for the amylose fraction in the chromatogram for the normal corn, was made (plot not shown). The sample was vortexed for 5 min. The slope of the plot was 0.57, which was greater than that in water (0.39) measured in preliminary experiment. A similar trend was reported by Roger et al. (2000) with synthetic amylose in water and KOH (0.52 and 0.62, respectively). The result proves that amylose has a fairly rigid structure in water, as reported by Hanselmann et al. (1995), but is more expanded in alkaline solution.

3.2. Molecular characteristics of waxy corn starch

Table 2 shows the structural analysis data for the waxy corn starch dissolved in 1 M NaOH at different vortexing times (up to 20 min). Even after 20 min vortexing, starch solubility remained less than 90%. But there were noticeable increases in M_w and solubility (87.6×10^6–185×10^6 g/mol, and 70.2–86.1%, respectively) by

Table 2
Average molecular weight (M_w), radius of gyration (R_g) and solubility of waxy corn starches dissolved in 1 M NaOH at different vortexing periods

Vortexing (min)	M_w ($\times 10^6$ g/mol)	R_g (nm)	Solubility (%)
02	87.6 ± 1.3	208 ± 0.3	70.2 ± 1.2
10	145 ± 1.4	198 ± 0.4	78.5 ± 2.2
15	185 ± 1.0	214 ± 0.4	86.1 ± 0.9
20	166 ± 1.2	215 ± 0.4	89.4 ± 0.8

Values are average of three replicates of each sample.

increasing vortexing time from 2 to 15 min. The changes were from the solubilization of the large M_w amylopectin chains. The M_w was decreased by 20 min vortexing, indicating molecular degradation. To dissolve waxy starch completely, more mechanical forces (shearing) is required in comparison to normal starch, but in both starches, excess force (20 min vortexing in this case) induces molecular degradation.

The molecular changes, caused by the vortexing and shown by the chromatogram, was somewhat different from those for normal corn starches (Fig. 2. vs. Fig. 1). In normal corn starch, chromatographic separation between amylopectin and amylose should be done prior to structural characterization of each polymer. Thus not only dissolution of amylopectin but also the dissociation of amylose from amylopectin should be considered. As shown in Fig. 2, with 2 min vortexing, amylopectin eluted as a broad peak, but with 10 min vortexing, the amylopectin peak appeared at lower elution volumes and it showed a shoulder. The light scattering detector showed a clear difference in M_w between the front peak and the shoulder (data not shown), indicating that the shoulder could be a different fraction from the amylopectin peak. At 15 min vortexing, the shoulder remained, but at 20 min, a broadened peak was appeared (Fig. 2). The chromatogram revealed that molecular degradation was clearly observed when starch was vortexed

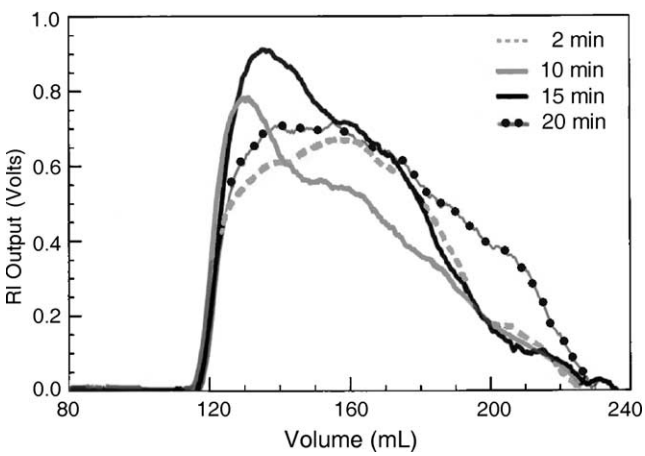

Fig. 2. Refractive index chromatograms of waxy corn starches dissolved in 1 M NaOH at different vortexing periods.

Table 3
Average molecular weight (M_w), radius of gyration (R_g) and solubility of high amylose corn starches dissolved in 1 M NaOH at different vortexing periods

Vortexing (min)	M_w ($\times 10^6$ g/mol)		R_g (nm)		Amylose content (%)	Solubility (%)
	Amylopectin	Amylose	Amylopectin	Amylose		
02	101 ± 2.1	2.5 ± 1.3	184 ± 0.5	89.0 ± 2.4	94.7	90.1 ± 1.2
05	113 ± 0.9	2.7 ± 1.3	175 ± 0.3	99.3 ± 0.8	80.1	95.2 ± 0.5
10	46.5 ± 0.9	2.1 ± 1.3	211 ± 0.3	81.6 ± 0.6	85.7	96.7 ± 0.8

Values are average of three replicates of each sample.

for 20 min. Because M_w continued to increase with vortexing time from 2 to 15 min (Table 2), the shoulder amylopectin could not be from degraded fragments of amylopectin. In the SEC analysis commonly practiced, amylopectin elutes at the void volume of the column as a sharp peak. However, the SEC column used in this study had a relatively wide range for M_w analysis (100,000–10,000,000), so some amylopectin eluted in its analytical range. The presence of the chromatographic shoulder may suggest that a low M_w amylopectin fraction actually exists appearing as a separate fraction.

Overall chromatograms showed that vortexing for 15 min could be an optimal treatment to dissolve amylopectin in 1 M NaOH for SEC analysis, although the starch dissolution was not complete.

3.3. Molecular characteristics of high amylose corn starch

Table 3 shows the molecular characteristics of the high amylose corn starches dissolved in 1 M NaOH by vortexing different periods at room temperature. When vortexed for 2 min, the starch exhibited amylopectin and amylose peaks with M_w 101×10^6 and 2.5×10^6 g/mol, respectively, and starch solubility of 90.1%. But the amylose content measured from the RI chromatogram was high (94.7%). When vortexing time increased to 5 min, the solubility and the M_w of both amylopectin and amylose increased (95.2%, 113×10^6, and 2.7×10^6, respectively). By 10 min vortexing, the M_w of both starch molecules was significantly decreased (46.5×10^6 and 2.1×10^6, respectively), showing degradation. Among the three corn starches tested, high amylose corn starch gave the greatest solubility at the same vortexing time although the molecular degradation was most substantial. This was because, as suggested previously, amylopectin was more difficult to dissolve, and less susceptible to the physical treatment.

Gérard, Barron, Colonna and Planchot (2001) reported that amylose extender (ae) starch was composed of intermediate starches, and thus amylose content as determined by SEC was difficult because of the presence of intermediates. In this study, we could not separate the intermediate fraction, and the amylose content in high amylose corn starch was higher than that commonly known for the starch (~70%). As shown in the RI chromatograms (Fig. 3), the amylose peak was partially overlapped by the salt peak due to the limit in the analytical range of the column. The high amylose content (>80%) measured in this experiment might be also due to inaccurate measurement from the chromatogram.

The specific volume of gyration (SV_g) from the M_w and R_g values was calculated for amylopectin and amylose, under an assumption that the molecules had spherical conformation (You & Lim, 2000). This value provides theoretical gyration volume per unit molar mass. The average SV_g of amylopectin chains was not same in the three corn starches tested under optimum conditions (vortexing 15, 5, and 5 min, respectively): waxy corn amylopectin 0.17; normal corn amylopectin 0.26; high amylose corn amylopectin; 0.33. The difference in SV_g suggests that those corn starches have amylopectin chains different in their structure, possibly in degree of branching and chain length. Taki, Suzuki, Taki, Hisamatsu and Yamada (1988) suggested that amylopectin from waxy corn starch was different from that of normal or high amylose corn starch, on the basis of the fractionation behavior in aqueous 1-butanol. It has been reported that amylopectin in waxy starch had a larger M_w than that in normal starch. Our results (Tables 1 and 2) revealed the same trend. The M_w of amylopectin was

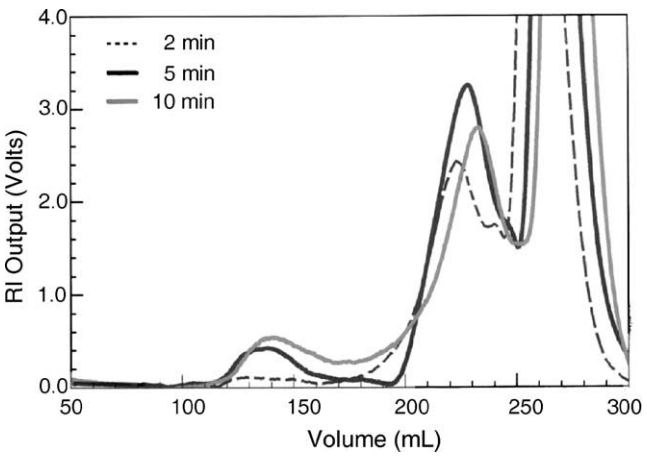

Fig. 3. Refractive index chromatograms of high amylose corn starches dissolved in 1 M NaOH at different vortexing periods.

decreased as the amylose content in the starch increased. Amylopectin of shorter chains and shorter distance between successive chains can produce more densely packed amylopectin structure (Gérard et al., 2001). It was also found that the long B chains in waxy corn amylopectin were much shorter than those in normal corn amylopectin, and high amylose corn amylopectin had the longest B chains (Han, BeMiller, Hamaker, & Lim, 2003). Therefore, the longer B chains resulted in the higher SV_g value as shown in the result.

4. Conclusions

The physical treatment for starch dissolution in alkaline solution, such as vortexing in the present study, is required to maximize starch solubility, but it should be carefully controlled to minimize chain degradations. The susceptibility of starch molecules to the treatment depends on the starch structure, so that amylose and amylopectin composition should also be considered. These results suggested that the presence of amylopectin made the dry amorphous starch more difficult to dissolve in alkaline solution. For accurate analysis with SEC, optimization of starch dissolution is suggested particularly when an alkaline solution is used.

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