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Rice Starch, Amylopectin, and Amylose: Molecular Weight and Solubility in Dimethyl Sulfoxide-Based Solvents

FANG ZHONG,† WALLACE YOKOYAMA,‡ QIAN WANG,† AND CHARLES F. SHOEMAKER*,§

School of Food Science and Technology, Southern Yangtze University, Wuxi, China, Western Regional Research Center, ARS, USDA, Albany, California 94710, and Department of Food Science and Technology, University of California, Davis, California 95616

Dimethyl sulfoxide (DMSO), with either 50 mM LiBr, 10% water, or both, was used as solvent for multi-angle laser-light scattering (MALLS) batch mode analysis of rice starch, and amylopectin and amylose weight-average molecular weight (M_w). DMSO/50 mM LiBr was a better solvent for these measurements than was DMSO/10% water, based on this solvent's ability to dissolve starch and to reduce the size of starch aggregates. Starch concentration decreased and amylose:amylopectin ratio increased when starch suspended in DMSO was centrifuged or filtered prior to size-exclusion chromatography (SEC)-MALLS analysis. A higher amylose:amylopectin ratio made starch more soluble, and the higher this ratio, the lower the M_w of eluted amylopectin. For SEC analysis of M_w, fractions of starch amylopectin and amylose dispersed in DMSO-based solvents yielded better results than starch dispersed directly into the solvents, because dispersion of these fractions decreased starch aggregation. When these two starch components were fractionated and then dissolved separately in DMSO/50 mM LiBr, the M_w of dispersed amylopectin ranged from 40 to 50 million, and that of amylose was ca. 3 million, whereas starch from three rice varieties of varying amylose content ranged from 60 to 130 million. We recommend that SEC evaluation of amylopectin and amylose be accomplished with fractionated samples as in this study; such evaluations were superior to evaluations of natural mixtures of amylopectin and amylose.

KEYWORDS: Starch; rice; amylopectin; amylose; starch molar mass; multi-angle laser-light scattering; size-exclusion chromatography

INTRODUCTION

Starch is widely used as a functional component in prepared foods and is the major source of caloric energy for most humans and domestic animals. Understanding the relationship of starch functionality to its fundamental molecular properties, such as weight-averaged molecular weight (M_w) and structure, has long been a goal of food scientists. Starch is composed of the glucose polymers amylopectin and amylose. The characteristics of foods containing starch are understood to be due largely to the mass ratio of amylose:amylopectin and the M_w of amylose (*1*−*3*). The pasting peak viscosity and breakdown viscosity of wheat and rice starch were negatively correlated with amylose content (*2*, *3*). Long chain-length branches of amylopectin and intermediate size branches of amylose produced the greatest synergistic effect on pasting viscosity of reconstituted starch (*4*). The role of amylopectin size in starch functionality has been difficult to determine because of its tendency to form insoluble aggregates. A key part of the picture of starch functionality includes sound data on the unassociated molecular structure of all starch polymers. The fundamental starting point is the knowledge of the M_w and size of amylose and amylopectin starch molecules.

The M_w of polymers is commonly determined by size-exclusion chromatography (SEC). However, this measurement for starch is challenging because calibration standards are usually necessary, and the highest M_w calibration polymer standard available is 2 million. This is significantly lower than the M_w of amylopectin. In recent years, high-pressure (HP) SEC instrumentation equipped with both MALLS instrumentation and differential refractometer (RI) has been used routinely to determine the M_w of polymers without the use of standards. This technique makes starch M_w measurement possible (*5*−*7*), but to obtain the accurate M_w's of amylopectin or amylose by this technique, the complete dissolution of amylopectin and amylose is necessary.

Dissolving starch is a minimum requirement for the separation and M_w determination of amylopectin and amylose by HPSEC. Soluble but entangled amylose and amylopectin will lead to M_w values higher than their true values. The limited solubility

* Author to whom correspondence should be addressed [telephone (530) 752-7347; fax (530) 752-4759; e-mail cfshoemaker@ucdavis.edu].
† Southern Yangtze University.
‡ USDA.
§ University of California.

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of starch in neutral aqueous solution makes structural analysis of starch in aqueous media difficult (*8*). High temperatures and high pH increase the solubility of many cereal starches in aqueous solvents, but may result in molecular size reduction resulting from degradation, depolymerization, or oxidation (*9*). Dimethyl sulfoxide (DMSO) is the most frequently used polar aprotic solvent for SEC analysis (*10*). DMSO disperses starch by acting as a hydrogen bond acceptor, disrupting inter- and intramolecular starch−starch and water−starch hydrogen bonds, and replaces hydroxyl−hydroxyl hydrogen bonding with DMSO−starch hydrogen bonding (*11*). To increase the solubility of starch in DMSO, addition of small amounts of water (*12*) or low M_w electrolytes (*9*, *13*, *14*) can be used. Jackson (*10*) measured the effect of DMSO:water ratio on cornstarch solubility and concluded that maximum dispersibility was obtained in a DMSO:water (9:1, v/v) solution; this solution was used in many subsequent starch structure analyses (*1*, *8*, *15*−*17*).

Large differences (up to 20-fold) in M_w of waxy cornstarch were reported by different investigators using 90% DMSO as a solvent (*8*, *15*, *18*, *19*). Mua and Jackson (*19*) measured the weight average M_w of waxy corn amylopectin isolated by aqueous leaching; they reported a M_w of 0.39×10^8. After filtering a DSMO−water starch solution through a 5.0-μm filter, the M_w of waxy corn amylopectin was 2.54×10^8 (*8*), but that of directly injected unfiltered waxy cornstarch in 90% DMSO was 7.5×10^8 (*15*). Variable solubilization of starch and the existence of amylopectin−amylopectin or amylose−amylopectin aggregates could cause this variation in reported M_w. Other investigators who used salts, including LiBr and $NaNO_3$, instead of water in DMSO (*9*, *13*) reported variation in the M_w of cornstarches.

In the present study, starch dispersibility in 90% DMSO and DMSO/50 mM LiBr (LBDMSO) solvents was compared by measuring starch solubility and MALLS batch-mode M_w of three types of rice starch, a waxy, low-amylose starch, a medium-grain starch, and a long-grain starch. Influence of starch sample preparation methods on the MW distribution profiles was investigated with a HPSEC-MALLS-RI system. The M_w distribution profiles of amylopectin and amylose in the three intact starches were compared to those of purified amylopectin, amylose, and their mixtures after their separation by aqueous leaching.

MATERIALS AND METHODS

Samples. Waxy (Calmochi 101, CMC), low-amylose (BL-1), medium-grain (M202), and long-grain (Cocodrie, CCD) rice samples were provided by the California Cooperative Rice Research Foundation, Biggs, CA. Starch was isolated using Pronase, an alkaline protease from *Streptomyces griseus*, according to the method developed by Biliaderis and Juliano (*20*).

Starch Solubility in DMSO-Based Solvents. A 20-mg sample of isolated starch was dispersed in either 5 mL of 50 mM LiBr in DMSO (HPLC grade, Sigma Chemical Co., St. Louis, MO), 90% DMSO, or 50 mM LiBr in 90% DMSO at 90 °C for 2 h on a stirrer-heater module (Prince Chemical Co., Rockford, IL) followed by stirring for 24 h using a magnetic stirrer at room temperature. Dispersed samples were centrifuged for 10 min at 13 500*g*. Total starch in the suspension before centrifugation and in the supernatant after centrifugation was measured enzymatically using a total starch kit (Megazyme, Wicklow, Ireland).

Preparation of Starch Solutions for MALLS Batch Mode M_w Measurement. Starch dispersions in 50 mM LiBr in DMSO, 90% DMSO, or 50 mM LiBr in 90% DMSO were prepared as described above. After centrifugation, the supernatants were retained and their starch concentrations were measured with the total starch kit. Each supernatant sample was then used for dilutions to five different starch concentrations in the range of 0.02−0.2 mg/mL. Solvents used for the dispersions were filtered through 1.2-μm nylon syringe filters.

Measurement of Starch M_w by MALLS Batch Mode. Diluted starch dispersions, with concentrations from 0.02 to 0.2 mg/mL, were injected with a manual injector (2 mL injection loop) directly to a MALLS detector (Dawn DSP-F, Wyatt Technology Corp., Santa Barbara, CA) at a flow rate of 0.5 mL/min. The laser source used was He−Ne, $\lambda = 623.8$ nm with a K-5 flow cell. Flow rate was controlled by a Shimadzu LC-10AD pump (Shimadzu Scientific Instruments, Inc., Kyoto, Japan), and a PLgel Mixed-A (Polymer Laboratories, Amherst, MA) column was connected between the pump and manual injector to provide backpressure for the HPLC pump. The solvent used to deliver the starch dispersion to the flow cell was the same as the solvent used to prepare the individual starch dispersions. For each starch sample, light scattering intensities were collected at 18 angles for each concentration. These data were analyzed by Astra software Batch method (version 3.4, Wyatt Technologies, Santa Barbara, CA). This analysis follows the Zimm treatment (*21*) of the light scattering parameters as a function of scattering angle and concentration. A second-order Berry equation (*22*) with an angle fit degree of 2 was used to fit each of the curves for the computer generated Zimm plot. The starch M_w and *z*-averaged mean square radius (RMS) were calculated by the Zimm treatment, which extrapolated the family of curves to obtained these values.

Preparation of Starch Dispersions for the HPSEC-MALLS-RI System. Ten preparations of M202 rice starch were dispersed in LBDMSO at a concentration of 0.4% (w/v). Six of the samples were preheated at 90 °C for either 15 min, 30 min, 1 h, 2 h, 4 h, or 8 h. Another set of starch dispersions was prepared by heating the dispersions at 90, 100, 110, or 120 °C for 2 h with a stirrer-heater module. After each treatment, the dispersion was continuously stirred at room temperature for 24 h. The starch dispersions were then centrifuged at 13 500*g*, and the supernatants were analyzed with the HPSEC-MALLS-RI system.

SEC analysis of 0.4% CMC, M202, and CCD rice starch dispersions were heated at 90 °C for 2 h and injected into the SEC system after either no further treatment (control), centrifugation at 13 500*g*, or filtration through a 1.2-μm nylon syringe filter. The exact amylose content of the samples prepared for injection was analyzed with an amylose−amylopectin assay kit (Megazyme) based on the concanavalin A method (*23*).

HPSEC-MALLS-RI System. The HPSEC-MALLS system consisted of an HP1050 series pump, an HP1050 autoinjector (Hewlett-Packard, Valley Forge, PA), a MALLS detector (Dawn DSP-F, Wyatt Technologies, Santa Barbara, CA), and a differential refractometer detector (ERC-7512, ERMA Inc., Tokyo, Japan). For the SEC analysis, three Styragel guard columns, Styragel HMW7, Styragel HMW6, and Styragel HMW2 (Styragel, Waters, Milford, MA), were connected in series, and LBDMSO was used as mobile phase at a flow rate of 0.6 mL/min. The Styragel HMW columns have high-porosity 10 μm frits and 20 μm particles for analysis of ultrahigh molecular weight polymers that are susceptible to shearing. Column temperature was maintained at 40 °C with a column heater (Bio-Rad, Hercules, CA).

Data Treatment. SEC data were analyzed by Astra software (version 3.4, Wyatt Technologies). A second-order Berry method was used for curve fitting. MW calculations were based on the mobile phase refractive index of 1.4785 and the d*n*/d*c* value of 0.066.

Separation of Amylose and Amylopectin by Aqueous Leaching. Aqueous leaching of amylose from isolated starch was carried out according to the method reported by Mua and Jackson (*24*), with some modifications. An amylose-rich fraction was leached from 1% (W/V) BL-1, M202, or CCD starch slurries by gentle stirring for 1 h at 65, 70, and 75 °C, respectively. The samples were then centrifuged at 5000*g* for 10 min; the supernatants were collected and the residues were leached again under the same conditions. After the second leaching and centrifuging, the first and second supernatant fractions were combined, and 100% *n*-butanol (at one-third the volume of supernatant) was added. The mixture was stirred and held at room temperature for 4 h before centrifuging for 10 min at 5000*g* to precipitate amylose. Slurries were again prepared from the residues, washed with water twice, and centrifuged. The final residues were mixed with methanol

Table 1. Solubility Index of Waxy and Nonwaxy Rice Starches in Three DMSO-Based Solvents after Centrifugation

rice starch	amylose content	starch solubility index (%)[a,b] DMSO/ 50 mM LiBr	90% DMSO/10% water/50 mM LiBr	90% DMSO/ 10% water
CMC	1.0%	75.0 ± 1.1	60.1 ± 2.0	49.1 ± 1.5
M202	14.2%	81.2 ± 1.7	68.4 ± 1.4	66.1 ± 1.0
CCD	20.1%	87.4 ± 1.4	75.5 ± 1.1	70.4 ± 1.7

[a] Solubility index is the percentage of total starch in supernatant after 10 min centrifuge at 13 500*g*. [b] Solubility index values are mean values of three analyses.

and centrifuged to precipitate amylopectin. The fractionated amylose and amylopectin were freeze-dried. Purity of the separated amylose and amylopectin was tested using the amylose−amylopectin assay kit. For SEC analysis, amylose and amylopectin samples were dissolved in DMSO/50 mM LiBr. The dispersions were heated at 90 °C for 2 h and, after cooling, were stirred for 24 h at room temperature. The dispersions were centrifuged at 13 500*g* for 10 min, and the supernatants were retained for SEC-MALLS analysis.

RESULTS AND DISCUSSION

Solubility of Waxy and Nonwaxy Rice Starches in DMSO-Based Solvents. The solubility of waxy and nonwaxy starches in various solvents and the use of different methods to separate insoluble starches or aggregates have been reported. These methods include centrifugation (*25*−*27*), filtration (*8*, *28*), and measurement of mass after elution from HPSEC (*6*, *28*). Selected centrifuge speeds varied from 2200 to 50 740 rpm, and filter pore sizes varied from 0.45 to 5.0 μm. Thus, the reported starch solubilities in different solvents and studies are difficult to compare. In the present study, the solubilities of waxy, medium-grain, and long-grain rice starches in three DMSO-based solvents were determined enzymatically after centrifugation at 13 500*g* for 10 min (**Table 1**). Based on the amount of starch in the supernatant after centrifugation, the best solvent was LBDMSO. Water added alone to the DMSO (90% DMSO) was the poorest solvent tested. For the waxy starch, CMC, the solubility in LBDMSO and in 90% DMSO was 75.0% and 49.1%, respectively. Rice starch with higher amylose content was more soluble than that with a lower amylose content in each of the three solvents tested, in agreement with reports of DMSO solubility with cornstarch (*10*) or starch from other sources (*9*).

Table 2. Batch Mode M_w and RMS of Rice Starches in DMSO-Based Solvents[a,b]

solvent	rice variety	$M_w \times 10^8$	RMS (nm)
DMSO/50 mM LiBr	CMC	3.62 ± 0.38	271.3 ± 11.3
	M202	2.53 ± 0.28	285.7 ± 16.6
	CCD	1.64 ± 0.13	252.6 ± 7.1
90% DMSO/10% water/ 50 mM LiBr	CMC	15.8 ± 1.2	426.9 ± 13.0
	M202	8.50 ± 0.78	504.8 ± 20.3
	CCD	3.73 ± 0.20	334.8 ± 5.0
90% DMSO/10% water	CMC	15.87 ± 1.40	492.0 ± 12.1
	M202	9.63 ± 0.79	486.0 ± 16.0
	CCD	4.00 ± 0.18	336.6 ± 5.0

[a] Starch was dispersed in solvent by stirring and heating at 90 °C for 2 h and then stirring for 24 h at room temperature. [b] Starch solutions at five concentrations, 0.02–0.2 mg/mL, were used for M_w and RMS determination.

Batch Measurement of Rice Starches M_w in DMSO-Based Solvents. Whereas the solubility of waxy and nonwaxy rice starches based on centrifugation was higher in LBDMSO than in either 90% DMSO or 50 mM LiBr in 90% DMSO, the ability of these solvents to minimize starch molecular aggregation was explored. Low HPSEC recovery values reported by Jackson (*10*) indicated that polymer aggregates may exist in starch dispersions after centrifugation, and these aggregates could be retained by HPSEC columns. To get a sense of the degree of starch aggregation in LBDMSO, 50 mM LiBr in 90% DMSO, and 90% DMSO, the dispersed states of rice starch in these solvents were characterized by MALLS batch mode analysis. The Zimm graphic method was used to determine the M_w and RMS of the dispersed starch. An example of the graphic treatment of a starch sample is presented in **Figure 1**, and M_w and RMS results are summarized in **Table 2**. Millard et al. (*15*) reported that the best fitting lines for extrapolation to the zero scattering angle and concentration were achieved using second or third degree scattering angle fit and zero or first degree concentration fit. For our analysis of the light scattering data, the lowest angles (detectors 1−13) of the 18-angle detector were used. The Berry equation with a second-order scattering angle fit and a first-order concentration fit was used for curve fitting.

M_w and RMS for each rice starch were similar in 90% DMSO and 50 mM LiBr in 90% DMSO, whereas the corresponding values in LBDMSO were lower. The lower values in LBDMSO suggest less aggregation of the starch molecules in this solvent.

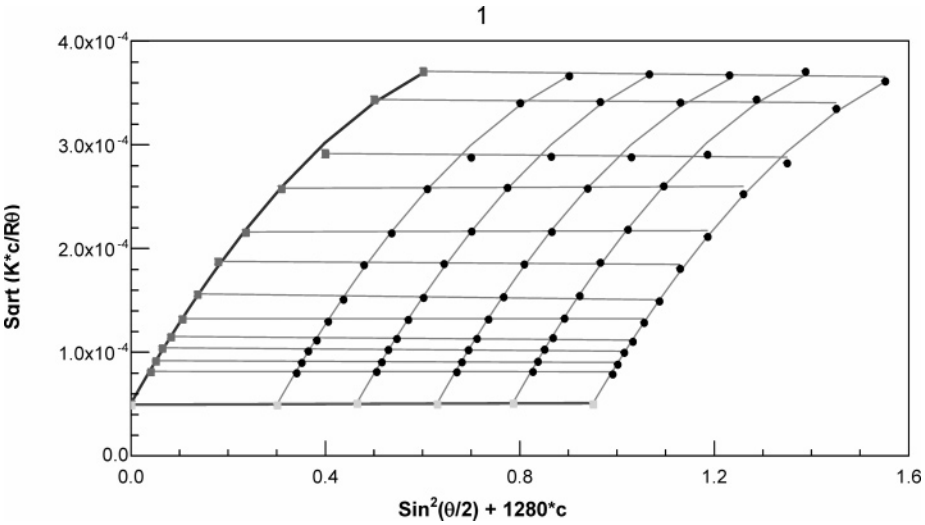

Figure 1. MALLS batch mode graphical analysis of CCD starch dissolved in 90% DMSO. The curves were fitted to the points with a second-order Berry equation with angle fit degree 2 and concentration fit degree 1. Detectors at the low angles (detectors 1–13) were used. The Zimm method was used to determine the M_w and RMS values.

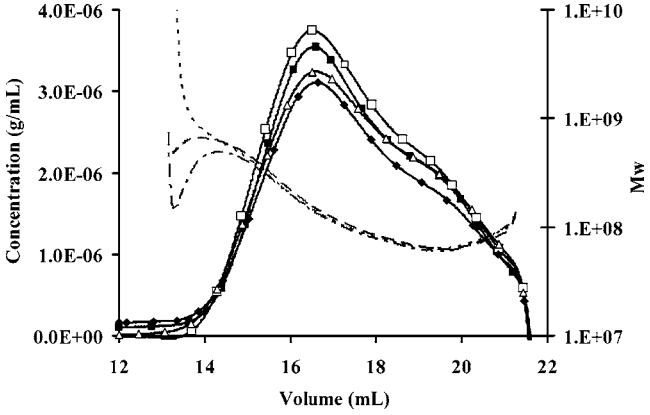

Figure 2. MW distributions of M202 starch in LBDMSO after heating at 90 °C for different time intervals as determined by the HPSEC-MALLS-RI system. Heating times were 1 h (■) 2 h (□), 4 h (△), and 8 h (◆). The four MW profiles are shown as dashed lines.

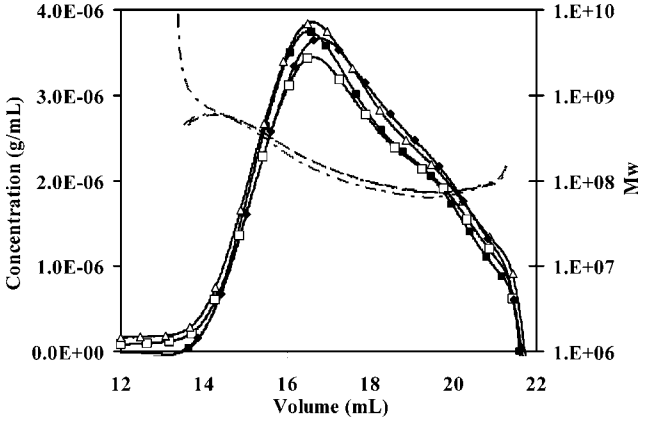

Figure 3. MW distribution of M202 starch in LBDMSO after heating at different temperatures for 30 min as determined by the HPSEC-MALLS-RI system. Heating temperatures were 90 °C (■), 100 °C (□), 110 °C (△), and 120 °C (◆). The four MW profiles are shown as dashed lines.

In each solvent, the M_w of the starch in the three rice samples followed the same trend, the higher the amylose content, the lower the M_w. The M_w differences among the starches dispersed in the three solvents were greater in waxy rice starch than in the medium-grain and long-grain starches. The M_w of CMC starch in 90% DMSO was 4.37-fold greater than in LBDMSO, but the difference between the M_w of CCD in these two solvents was 2.43-fold. The results suggested more favorable intragranular conditions for aggregation of amylopectin in low-amylose-containing starches. The M_w and RMS data for rice amylopectin obtained by batch measurement in this study were much lower than those reported by Yoo and Jane (*1*), who work with aqueous solvents. The M_w of amylopectin from waxy rice and nonwaxy rice was 56.8×10^8 and 26.8×10^8, respectively. Because distilled−deionized water was used as the starch solvent and HPSEC mobile phase in the analyses of Yoo and Jane (*1*), their results suggest that larger aggregates of starch polymers are created in aqueous dispersions.

Effect of Starch Dispersion Heating Time and Temperature on M_w. The effectiveness of different heating temperature and time of heating to disperse M202 rice starch in LBDMSO was determined by measuring the M_w of solutions using a HPSEC-MALLS-RI system. Heating times ranged from 1 to 8 h at 90 °C (**Figure 2**), and heating temperatures ranged from 90 to 120 °C for a 2-h period (**Figure 3**). Profiles of M_w plotted against elution volume were almost identical among samples, but starch solubilities, as determined by eluted mass, among the different heating times were different. The eluted mass was greatest when starch was dispersed by stirring and heating at 90 °C for 2 h (data not shown). Jackson (*10*) reported that starch recovery from 90% DMSO dispersions increased with prolonged heating time, from 18 to 89 h, at 90 °C. In their study, the dispersed starch was centrifuged at 3000*g* as compared to 13 500*g* used in the present study to recover the dispersed starch. Jackson (*10*) did not report the M_w's of their samples.

There were no differences in the SEC RI profiles among dispersions prepared at 90−120 °C for 2 h (**Figure 3**), which suggests that no significant differences in solubility, changes in molecular structure, or aggregation resulted from heating temperature in this temperature range when samples were dispersed in LBDMSO.

Effect of Preparation Methods of Starch Dispersions for SEC Analysis of MW. Rice starches were dispersed in LBDMSO, heated for 2 h at 90 °C, and stirred continuously for 24 h at room temperature, and the effect of either centrifugation or filtration of the starch dispersions before SEC analysis was determined. The three starches used were BL-1 (9% amylose), M202 (15% amylose), and CCD (20% amylose). Immediately after centrifugation or filtration of the starch dispersions, total starch and amylose content were measured before injection into the SEC system (**Table 3**). The composition of the dispersion was dependent on the treatment method. Filtration through a 1.2-μm filter removed starch aggregates more effectively than centrifugation at 13 500*g*, because filtration decreased starch concentration to a greater extent, and there were larger recoveries of HPSEC eluted mass. These results depended on the exact conditions of centrifugation and filtration, and the effects of these treatments on the original molecular distribution of the starch molecules in the rice starch dispersions required further M_w distribution analysis by SEC.

Both filtration and centrifugation decreased the starch content and increased the proportion of amylose in each of the three rice starch dispersions (**Table 3**). The increase in amylose concentration was proportional to the decrease of total soluble starch. The decreases in soluble starch and M_w suggest that the aggregates removed consisted primarily of amylopectin. The amount of starch removed by either treatment method was greatest for the starch containing the lowest amount of amylose, BL-1. Amylose appeared to aid in the dispersion of the rice starch by decreasing the tendency of amylopectin to aggregate. This was consistent with the results from the batch measurements made with different DMSO solvents (**Table 1**). Similar results were reported for corn (*8*, *24*) and barley starch (*16*). By plotting the starch M_w against amylose content, there was a negative linear relationship ($r = -0.991-0.997$) (**Figure 5**). This revealed that the lower M_w after centrifugation or filtration was correlated not only with the removal of polymer aggregates but also with an increase of amylose content as the M_w of amylopectin was about 100-fold higher than that of amylose (*8*, *16*, *24*).

As measured by SEC-MALLS analysis, the M_w and RMS values of M202 and CCD starch samples treated with centrifugation (**Table 3**) were lower than those obtained with batch mode MALLS measurements (**Table 2**) with the same treatment. The M_w values obtained by the batch mode were 2.5-fold higher than those obtained by SEC measurements, and the batch mode RMS values were about 100 nm higher than the SEC RMS values. This would occur if starch aggregates bypassed the centrifugation or filtration treatment and were retained by the SEC columns. Bath and Carlin (*29*) suggested that the degrada-

Table 3. Effect of Treatments of Rice Starch Dispersions on Concentration and Composition, and HPSEC Analysis of M_w and RMS[a,b]

variety	treatment	concentration[c] (%)	solubility index (%)[d]	amylose content (%)[e]	eluted mass (mg)[f]	mass recovery (%)[g]	$M_w \times 10^{-8}$ [h]	RMS (nm)[i]
BL-1	untreated[j]	0.34 ± 0.005	100	7.48 ± 0.23	0.202	58.5	1.40 (5%)[k]	200.4 (2%)
	centrifugation[l]	0.263 ± 0.004	76.0	9.03 ± 0.20	0.196	74.3	1.31 (5%)	191.4 (3%)
	filtration[m]	0.193 ± 0.002	55.8	14.08 ± 0.27	0.161	83.4	0.87 (5%)	154.8 (3%)
M202	untreated	0.348 ± 0.005	100	14.20 ± 0.26	0.251	72.1	1.08 (4%)	192.2 (3%)
	centrifugation	0.283 ± 0.006	81.2	16.7 ± 0.39	0.236	83.7	0.940 (5%)	181.7 (3%)
	filtration	0.243 ± 0.003	69.8	21.71 ± 0.21	0.209	85.9	0.711 (5%)	164.9 (3%)
CCD	untreated	0.348 ± 0.004	100	20.12 ± 0.27	0.271	77.9	0.702 (4%)	176.0 (3%)
	centrifugation	0.304 ± 0.005	87.4	23.54 ± 0.18	0.260	85.7	0.606 (5%)	173.2 (3%)
	filtration	0.255 ± 0.007	73.3	27.28 ± 0.31	0.237	93.1	0.460 (4%)	150.9 (3%)

[a] The solvent used for all samples was LBDMSO. [b] Data are means of at least two analyses. [c] Starch was tested enzymatically using a total starch kit. [d] Solubility index corresponds to the proportion (%) of total starch remaining suspended after centrifugation or filtration. [e] Amylose content was measured with the amylose kit. [f] HPSEC eluted mass was measured with a differential RI detector. [g] HPSEC recovery corresponds to the proportion (%) of injected starch recovered in the column effluent. [h] The M_w was determined from SEC-MALLS analysis. [i] *Z*-averaged molecular radius was determined from SEC-MALLS analysis. [j] Control sample dispersion was prepared by heating at 90 °C for 2 h and then stirring at room temperature for 24 h. [k] Error on curve fitting with MALLS. [l] Control sample that was further treated by centrifugation at 13 500*g* for 10 min. [m] Control sample was further filtered through a 1.2-μm nylon syringe filter.

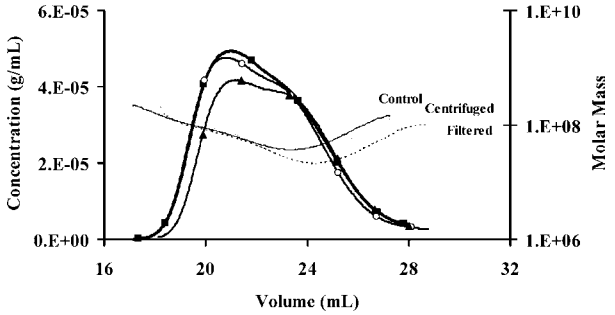

Figure 4. SEC chromatographs of CCD rice starch with different treatments before SEC-MALLS analysis: control (■), centrifuged (○), and filtered (▲). Lines without symbols represent M_w of samples as labeled.

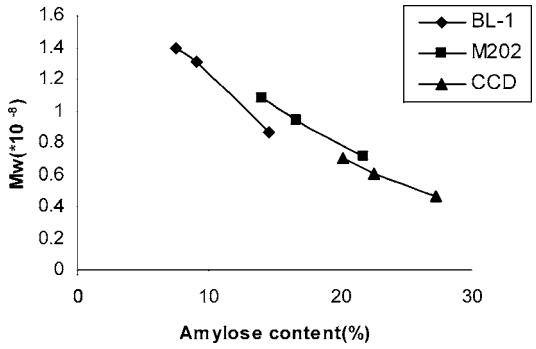

Figure 5. Relationship of rice starch MW to amylose content; amylose content was changed by centrifugation or filtration.

tion of starch aggregates by SEC columns could account for the lowering of the M_w. For the centrifuged samples, the recovery from the SEC analysis ranged from 74.3% to 85.7% (**Table 3**), and the SEC recoveries with the filtered samples were even higher. This suggests that prior to SEC analysis there were smaller amounts of aggregates in these treated dispersions as compared to untreated dispersions. For each of the three dispersed samples, the mass lost during SEC analysis may have been starch aggregates retained on the columns.

The SEC chromatographs for the CCD samples (**Figure 4**) showed a reduction for eluted mass with either centrifugation or filtration treatment, and there was also an overall reduction of M_w during the elution of these samples. This latter observation was unexpected for SEC analysis of the same types of samples with the same column bank. The shift in M_w profiles suggests that there was a steady background of high M_w molecules

Table 4. M_w, RMS, and Purity of Rice Amylopectin and Amylose[a–c]

rice varieties	M_w	RMS (nm)	HPSEC eluted mass (mg)[d]	purity (%)[e]
BL-1				
amylopectin	5.52×10^7 (5%)[f]	105.1 (6%)	0.321	96.3 ± 0.3
amylose	3.44×10^6 (6%)	103.3 (5%)	0.354	90.2 ± 0.4
M202				
amylopectin	4.64×10^7 (5%)	103.7 (7%)	0.354	95.9 ± 1.0
amylose	3.22×10^6 (4%)	95.6 (6%)	0.352	95.4 ± 0.3
CCD				
amylopectin	4.01×10^7 (5%)	98.9 (5%)	0.346	94.6 ± 0.6
amylose	3.12×10^6 (4%)	86.0 (7%)	0.355	94.8 ± 0.2

[a] Solvent used for all of the samples was LBDMSO. [b] Data are means of at least two analyses. [c] Samples were injected after heating, stirring, and centrifuging at 13 500*g* for 10 min. [d] Mass of starch eluted from HPSEC column; each injection mass was ca. 0.34–0.36 mg. [e] Purity was tested by the amylose–amylopectin assay kit. [f] Precision of polynomial fit.

flowing through the columns. This could have been the result of initial retention of amylopectin aggregates on the columns, followed by their gradual breakdown during the analysis, thus leading to a continuous introduction of amylopectin into the chromatographic flow. This would have produced M_w values that were higher than the actual values measured on a completely dispersed sample. An increase in M_w measured by SEC starch analysis was also reported by Yoo and Jane (*1*) and Yokoyama et al. (*9*).

***M*w Analysis of Rice Amylopectin and Amylose.** In the SEC analysis of nonwaxy starches, only two peaks were expected, those of amylopectin and amylose. However, there was only a main peak followed by an unresolved shoulder in the SEC profiles of the rice starches (**Figures 2−4**). The poor resolution could possibly be the result of the branched structure of amylopectin. This structure may be more compact than that of the linear amylose polymer, which may form a more rigid helical structure, thus making their sizes similar (**Table 4**). However, the incomplete molecular dispersion of amylopectin also hinders the potential for a better separation.

To address the problem of the poor SEC separation of rice amylopectin and amylose, rice starch was physically fractionated by aqueous leaching according to the method developed by Mua and Jackson (*19*, *24*). The M_w's of fractionated rice amylopectin and amylose were measured separately by HPSEC-MALLS, and the purity of the leached fractions was tested with the amylose−amylopectin kit (**Table 4**). The purity of each fraction ranged from 90% to 97%, which indicated good amylopectin and

Table 5. M_w and RMS of Recombined CCD Rice Amylopectin and Amylose[a]

amylopectin and amylose mixtures	MW	calculated M_w[b]	RMS (nm)	HPSEC eluted mass (mg)[c]
amylopectin	4.01×10^7 (5%)		98.9 (7%)	0.354
75% amylopectin + 25% amylose	3.11×10^7 (5%)	3.087×10^7	104.4 (6%)	0.359
50% amylopectin + 50% amylose	2.02×10^7 (5%)	2.162×10^7	101.3 (6%)	0.354
25% amylopectin + 75% amylose	9.78×10^7 (4%)	1.237×10^7	97.8 (6%)	0.351
amylose	3.12×10^6 (4%)		86 (6%)	0.352

[a] Amylopectin and amylose were heated and stirred in DMSO separately and then recombined at different ratios. Samples were injected after the mixtures were centrifuged at 13 500*g* for 10 min. [b] Calculated M_w = weight fraction of amylopectin*MW amylopectin + weight fraction of amylose*MW amylose. [c] Injected M_w of injected samples ranged from ca. 0.34 to 0.36 mg.

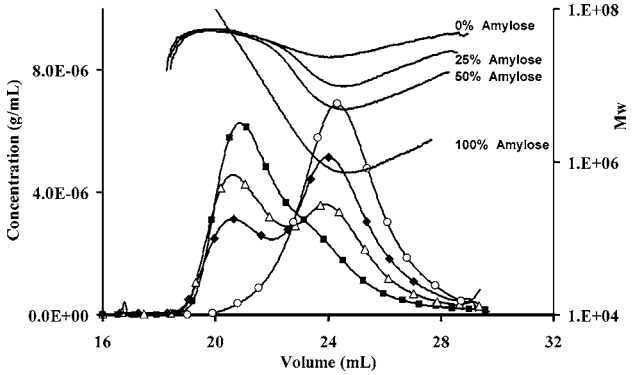

Figure 6. SEC chromatographs of mixed CCD amylopectin (AP) and amylose at different ratios, 0% amylose (■), 25% amylose (△), 50% amylose (◆), and 0% amylose (○), determined with a HPSEC-MALLS-RI system. The solid lines represent the M_w for each mixture as it eluted.

amylose fractionation by leaching. The M_w and RMS values of amylose from the three rice starches were around 3 million and 100 nm, respectively. These values are within the range of values reported by other researchers (*30*, *31*). The M_w and RMS of amylopectins, $(4.0-5.5) \times 10^7$ and 99−105 nm, respectively, were significantly lower than M_w and RMS values of whole starches (**Table 3**) and amylopectin reported by other researchers. There are many published values for amylopectin M_w in the 100−800 million range and RMS in the 200−500 nm range (*1*, *4*, *8*, *9*, *15*, *16*); exceptions are those reported by Mua and Jackson (*19*) and Fishman et al. (*6*). Mua and Jackson (*19*) measured the M_w of leached corn amylopectin and reported M_w values of 29−54 million, and Fishman reported a value for waxy maize of 24 million.

The eluted masses of amylopectin or amylose determined by SEC analysis indicated greater than 90% recovery for all leached samples. This high proportion of recovery suggested a minimum amount of aggregation of amylopectin or amylose in the sample dispersions. Amylose and amylopectin in the starch granule may have gelatinized upon heating and formed aggregates when starch was dispersed directly in LBDMSO. With aqueous leaching fractionation of amylose and amylopectin, the starch granules swell on heating and release amylose into the aqueous phase, and the swollen but ungelatinized amylopectin does not aggregate if the pasting peak temperature is not reached (*24*). Therefore, when the recovered amylopectin was dispersed in LBDMSO, the formation of heat-induced amylopectin aggregates was limited.

After leaching and dispersing in LBDMSO, the amylopectin and amylose dispersions were mixed in different ratios at room temperature and the M_w distribution profiles were measured by HPSEC-MALLS (**Figure 6** and **Table 5**). Comparing the mixture of the leached amylopectin (75%) and amylose (25%) (**Figure 6**) to the CCD starch (20% amylose) dissolved directly in solvent (**Figure 4**), the resolution of amylopectin and amylose was improved. The M_w's of leached mixtures were close to the predicted M_w's (**Table 5**). These results suggested that once amylose and amylopectin were well dispersed in DMSO, amylopectin aggregates were not formed during the mixing of the dispersions. However, as can be seen from the SEC chromatograph for the fractionated amylopectin, it still eluted into the elutation volume of amylose (**Figure 6**), making the resolution of amylopectin and amylose difficult even when aggregation was minimized.

This investigation showed the difficulty in obtaining true molecular dispersions of starch molecules. These results suggest that many previously reported M_w values of rice starch amylopectin and amylose may be an order of magnitude higher than the actual M_w values. Based on this study, evaluation of amylopectin and amylose M_w by SEC is recommended as being best accomplished with fractionated samples rather than with the natural mixture of amylopectin and amylose in starch.

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