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Sweet potato amylose and amylopectin with narrower distribution of molar mass and chain length obtained by a repeated retrogradation–hydrolysis procedure

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ABSTRACT: The wide molar mass distribution of native starch creates obstacles in investigating the physicochemical characteristics of starch, such as retrogradation, because samples thought to be the same are actually compounds containing many chains with different molar masses. In this paper, the sweet potato amylose and amylopectin isolated from retrograded starch were treated with the retrogradation–hydrolysis method three times, and their physicochemical changes in this process were determined by absorbance of the starch–iodine complex, light microscopy, and molar mass and chain length distributions. The results showed that repeated retrogradation and hydrolysis caused the molar mass distribution of sweet potato amylose and amylopectin to reduce from 4.2×10^7–205 and 7971–223 to 6.0×10^4–730 and 4533–211 g mol^{-1}, respectively. This retrogradation–hydrolysis cut the chain length distribution of sweet potato amylose from DP 9–35 to DP 3–13, but that of amylopectin remained unchanged. The double helix in sweet potato amylopectin will not form if the percentage of chain length with DP ≥ 4 is less than 25%. Repeated retrogradation and hydrolysis was an appropriate method to obtain amylose or amylopectin with a narrower molar mass distribution. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43849.

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INTRODUCTION

The preparation of single crystals with sharp X-ray diffraction peaks is a possible way to uncover the spatial structure of retrograded starch so as to have a deep insight into retrogradation. The wide molar mass distribution of retrograded starch is the greatest obstacle to obtaining such crystals and leads to wide X-ray diffraction peaks.[1,2] It is difficult to demonstrate the structure of retrograded starch only by such X-ray diffraction analyses because all of the cell parameters are uncertain. The chain lengths of amylose or amylopectin crystalline segments, prepared by hydrolysis of retrograded amylose or amylopectin, show a narrow distribution.[3,4] In other words, only those chains with a certain length could be involved in retrograded starch, so those chains with a narrower length distribution might be obtained by repeated retrogradation and hydrolysis.

According to the literature, the molar mass (MW) of sweet potato amylose, prepared by aqueous leaching, was found to be 5×10^5 g mol^{-1},[5] having relatively longer branches than that of other plants.[6] The molar mass of sweet potato amylopectin has been estimated to have a degree of polymerization (DP) of 9900 (about 1.6×10^6 g mol^{-1}) as measured by different techniques.[7,8] The chain length distribution of sweet potato amylopectin is 11% for DP 6–8, 27.9% for DP 9–12, 54.1% for DP 13–24, and 7.0% for DP 25–30.[9] But the chain length distributions of amylose and amylopectin in retrograded sweet potato starch are still unknown. It is suggested that one main factor

affecting the retrogradation rate of sweet potato starch is the proportion of short unit chains of amylopectin.[10] Lu *et al.*[11] indicated that the portion of short chains with DP 6–9 appeared to inhibit retrogradation. No retrogradation appeared to occur when the external chains of the amylopectin had 11 or fewer glucose units on average, and those very short external chains hindered the reassociation of the long external chains.[12–14] The contents of amylose and amylopectin in retrograded sweet potato starch are 76.4% and 23.6%, respectively.[15] The interaction between them has effects on starch retrogradation and brings more difficulties to a deeper understanding of retrogradation.[16–18] The entrapment of amorphous regions within imperfect crystals[19,20] in retrograded starches also makes it difficult to analyze the structure of retrograded starch. The distribution of MW and branched chains of starch greatly influences the crystal structure of starch.[21,22] Only those starches with a narrower molar mass distribution can be used to prepare crystals of retrograded starches with sharp peaks in X-ray diffraction,[23] so as to uncover the spatial structure of retrograded starch. Until now, such needle-like single crystals[24,25] and spherical crystals[26–28] have been prepared from short-chain amylose, and their X-ray diffraction peaks have been indexed according to the monoclinic unit cell or the hexagonal unit cell. In this paper, the sweet potato amylose and amylopectin in retrograded starch were isolated and treated with retrogradation and hydrolysis three times, and their physical changes in this process were determined by absorbance of the starch–iodine complex, infrared (IR), light microscopy, and molar mass and chain length distributions.

EXPERIMENTAL

Materials

Sweet potato starch (moisture content 19.3%, amylose content 23.6%) was provided by Shandong Jincheng Co. (Qingdao City, Shandong Province, China). Butanol and ethanol were purchased from Tianjin Fuyu Fine Chemical Co. (Tianjin City, China). Thermostable and midtemperature α-amylase (12,000 U/mL) from *Bacillus licheniformis* was purchased from Tianjin Nuoao Technology Development Limited Company (Tianjin City, China). Pullulanase (Enzyme Commission Number 3.2.1.41; 5000 U/mL), maltose, maltotriose, maltotetrose, maltopentaose, maltohexaose, and maltoheptaose were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Methods

Preparation and Isolation of Retrograded Sweet Potato Starches. The retrograded sweet potato starch was prepared according to our previous method.[15] First, 10 g of sweet potato starch blended with 100 mL distilled water was gelatinized for 20 min at 95 °C by continuous stirring. The gelatinized starches were autoclaved at 120 °C for 30 min and retrograded at 4 °C for 24 h. Then 0.6 mL high-temperature α-amylase (12,000 U/mL) was added to hydrolyze the unretrograded starch into soluble carbohydrates for 6 h at 90 °C. Crude retrograded sweet potato starch was precipitated by centrifuge (3040 × g for 5 min), and the supernatant fraction was discarded. The crude retrograded sweet potato starch was washed with deionized water three times to remove soluble impurities, and the purified retrograded starches were dried with hot air at the temperature of 55–60 °C in a drying oven to constant weight.[29]

Isolation of Amylose and Amylopectin in Retrograded Starches. The purified retrograded starches were dissolved in 4 *M* potassium hydroxide followed by neutralizing with 6 *M* hydrochloric acid. Then the amylose in retrograded sweet potato starch was precipitated from solution by adding three times the volume of 1-butanol, and further isolation was done by centrifuge (3040 × g for 5 min). The supernatants containing the amylopectin were collected, concentrated, and precipitated with excess ethanol. The amylopectin in the supernatants was isolated by centrifuge (3040 × g for 5 min). The drying method for the amylose and amylopectin was the same as that of purified retrograded starch in the previous section.

Repeated Retrogradation of Sweet Potato Amylose and Amylopectin. The repeatedly retrograded amylose and amylopectin samples derived from sweet potato amylose and amylopectin were prepared with the same treatment as the retrograded sweet potato starches; the only difference was that midtemperature α-amylase was used to purify the retrograded amylopectin.

Identification of the Wavelength Maxima. To identify the wavelength maxima (λ_{max}) of the samples, I_2/KI (1:3) solutions were prepared with a total of 5% (w/v) total iodine. First, 5.0 mg samples were dissolved in 4.0 *M* NaOH with magnetic stirring, and the pH of the solutions was adjusted to 7.0 by 6 *M* HCl. Then 0.25 mL I_2/KI (1:3) solutions were added rapidly, and the absorption spectra of the sample were recorded after 30 min using a UV-2501pc UV–vis recording spectrophotometer (Shimadzu, Japan).

IR Spectra. All samples were first dried at 120 °C for 2 h. Then each sample was mixed with potassium bromide (KBr) at the ratio of 1:60, and the mixture was compressed into slices by equipment. After that, samples were loaded onto a slide holder, and spectral curves were obtained in transmission mode using a Bio-Rad FES135 IR spectrometer (Bio-Rad Laboratories, Inc., Berkeley, California, USA) at 27 °C. The IR instrument was equipped with a deuterated triglycine sulfate detector using the digital attenuated total reflectance accessory at 4 cm^{-1} resolution for 64 scans.

Molar Mass Distribution Profiles. The molar mass distribution was determined by the method described by Philpot *et al.*[30] Sweet potato amylose or amylopectin (100 mg) was added to 80 mL of deionized water and heated in boiling water with stirring for 20 min to make the samples dissolve. After being filtered through 5 μm cellulose acetate filters, the dissolved samples were injected into a high-performance size-exclusion column chromatography system with a multiangle laser light scattering detector and a refractive index detector, a pump (LC-10AT, Shimadzu Corporation, Kyoto, Japan), an injector valve with a 100 μl loop (Rheodyne, Cotati, CA). Two series tandem columns (300 × 7.5 mm, PL aquagel-OH MIXED 8 μm, Polymer Laboratories Ltd., Shropshire, UK), a DAWN DSP-F laser photometer fitted with a He–Ne laser ($k = 623.8$ nm) with a K-5 flow cell (Wyatt Technology, Santa Barbara, CA), and an RID-

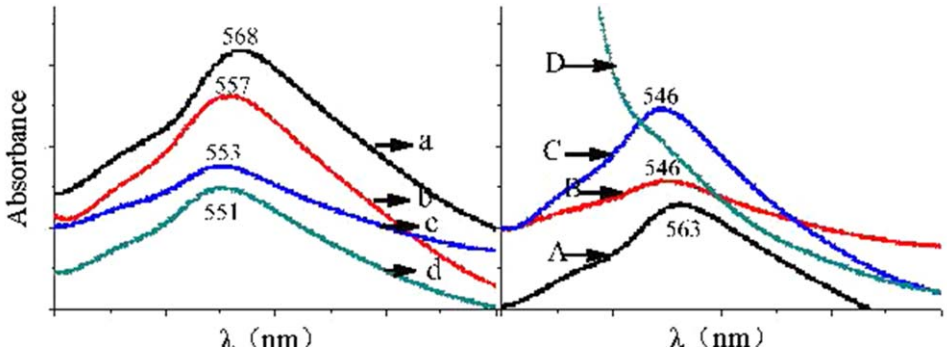

Figure 1. Visible spectra of retrograded sweet potato starches: (a–d) sweet potato amylose retrograded for one, two, three, and four times, respectively; (A–D) sweet potato amylopectin retrograded for one, two, three, and four times, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

10A differential refractive detector (Shimadzu Corporation) were used. The flow ratio was 0.5 mL/min. The mobile phase was a 0.1 *M* $NaNO_3$ solution containing 0.02% NaN_3, which had been passed through a 0.4 μm membrane filter (Membrane Solutions LLC, Osaka, Japan). A dn/dc value of 0.150 was used in the molar mass calculation (where dn is the difference between the refractive index, dc is the difference of the concentration, dn/dc value is a constant index for certain sample), and data handling was carried out using ASTRA software (Version 4.73.04) (Wyatt Technologies, Santa Barbara, CA).

Chain Length Distribution Profiles. The chain length distribution of sweet potato amylose or amylopectin was determined by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).[31] The samples (100 mg) were dissolved in 30 ml 4.0 *M* KOH, and the pH of the solutions was adjusted to 6.0 by 6.0 *M* HCl. Then pullulanase (0.5 U) was added to each solution for hydrolysis at 45 °C for 24 h with stirring. After that, the solution was heated in a boiling water bath for 10 min to deactivate the enzyme. The debranched sample solutions were first centrifuged at 21,500 × g and filtered through a 0.45 μm membrane filter before being injected into the HPAEC-PAD system. The HPAEC-PAD system included a Dionex DX 600 equipped with an ED 50 electrochemical detector with a gold working electrode, GP 50 gradient pump, LC 30 chromatography oven, and an AS40 automated sampler (Dionex Corporation, Sunnyvale, CA). The standard triple-potential waveform was used with the following period and pulse potentials: T1 = 0.40 s, with 0.20 s sampling time, E1 = 0.05 V; T2 = 0.20 s, E2 = 0.75 V; T3 = 0.40 s, E3 = −0.15 V. The data were collected using Chromeleon software (version 6.50, Dionex Corporation). The eluents were prepared in distilled deionized water with helium sparging; eluent A consisted of 200 m*M* NaOH, and eluent B consisted of 50 m*M* sodium acetate in 200 m*M* NaOH.

Optical Micrographs of Repeatedly Retrograded Sweet Potato Amylose and Amylopectin. Light microscopy photos were taken in an Olympus IX71 microscope (Olympus Corporation, Tokyo, Japan). A droplet of 4.0 *M* KOH solution was dripped onto the slide, and the wet starch was slowly immersed into it by an inoculating needle. Then the slide was dried at room temperature, and all optical micrographs were taken in 1000 × total magnification.

Statistical Analysis. Mean values from the three replicate experiments were reported. All samples were measured only once. The statistical significance of observed differences among treatment means was evaluated by analysis of variance (ANOVA) and a least significant difference test (Excel).

RESULTS AND DISCUSSION

The yields of retrograded sweet potato starch, amylose, and amylopectin are 21.5%, 17.2%, and 4.3%, respectively. Then the amylose and amylopectin are further retrograded one to three more times; the corresponding yields for retrograded amylose are 52.6%, 54.8%, and 64.3%, and for retrograded amylopectin, 6.6%, 4.8%, and 4.3%. These samples are purified and dried to conduct visible absorbance, IR, microphotography, and molar mass and chain length analyses.

Iodine Binding

The changes in the iodine binding properties of sweet potato amylose and amylopectin during repeated retrogradation are shown in Figure 1. The results show that the maximum absorption wavelength (λ_{max}) of the sweet potato amylose and amylopectin from the first retrogradation is 568 and 563 nm, respectively. These two wavelength maxima imply that sweet potato amylose and amylopectin probably interact with each other by way of forming double helices during retrogradation. Such results agree well with the literature,[32] except for the absence of λ_{max} higher than 600 nm, indicating that retrogradation and purification have cut the long chains down into short ones. After retrogradation four times, the λ_{max} of sweet potato amylose reduces to 551 nm and that of amylopectin disappears. The λ_{max} of starch attached with iodine originates from the double helix of starch, and the absence of it in retrograded amylopectin in the retrograded samples indicates that no double helix exists in it. So it is suggested that the double helix is not the indispensable condition for the occurrence of retrogradation of amylopectin.

Comparison of IR Spectra of Repeatedly Retrograded Sweet Potato Amylose and Amylopectin

The changes in the IR properties of sweet potato amylose and amylopectin during repeated retrogradation are shown in

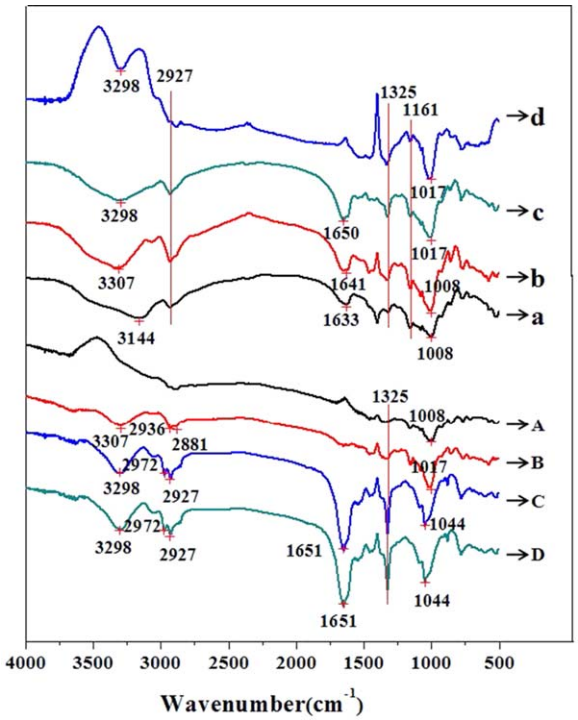

Figure 2. IR spectra of retrograded sweet potato starches: (a–d) sweet potato amyloses retrograded for one, two, three, and four times, respectively; (A–D) sweet potato retrograded amylopectins for one, two, three, and four times, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 2. For amylose in Figure 2(a–d), the obvious change in the IR spectra during repeated retrogradation is the reduction of the amplitudes in the absorption bands at approximately 1633 and 2927 cm^{-1}, which correspond to the bending vibration of O—H (water) and the asymmetric stretching vibration of methylene (starch).[33–35] This result suggests that the water content in retrograded sweet potato amylose decreases gradually, and more hydroxyls in the sixth carbon of amylose are involved in the formation of hydrogen bonds, so their effects on the asymmetric stretching vibration of C—H bonds in methylene are reduced. These changes also hint that more water probably combines with the sixth carbon atom of amylose in gelatinized starch. For sweet potato amylopectin in Figure 2(A–D), an adverse change in the IR spectra happens. The intensities of the bands at approximately 1651 and 2927 cm^{-1} are enhanced during repeated retrogradation, indicating that more water combines with amylopectin, and the breakage of 1,6-glycosidic bonds occurs to produce new methylene. The band at 2881 cm^{-1} in Figure 2(B) corresponds to the symmetric stretching vibration of methylene (starch), suggesting that the spatial structure of 1,6-glycosidic bonds begins to change before its breakage. The presence of the band at 2972 cm^{-1} in Figure 2(C,D) corresponds to the C—H stretching vibration of methyl; this may be caused by the attraction force of new hydrogen bonds between amylopectin. The reason for this should be further studied. The changes of other bands during repeated retrogradation are too complex to assign.

Microphotographs of Sweet Potato Amylose and Amylopectin during Repeated Retrogradation

Figure 3 shows the microphotographs of sweet potato amylose (a–d) and amylopectin (A–D) during repeated retrogradation and of the amylose (E) and amylopectin (F) bound together before the first retrogradation. Compared with the original amylose (E) and amylopectin (F), the obvious change in the samples is that the length of the branching chain is shortened from the first to the fourth retrogradation. The change implies that different sweet potato amyloses or amylopectins arrange themselves together side by side to form hydrogen bonds during retrogradation, and part of the branches in long chains not

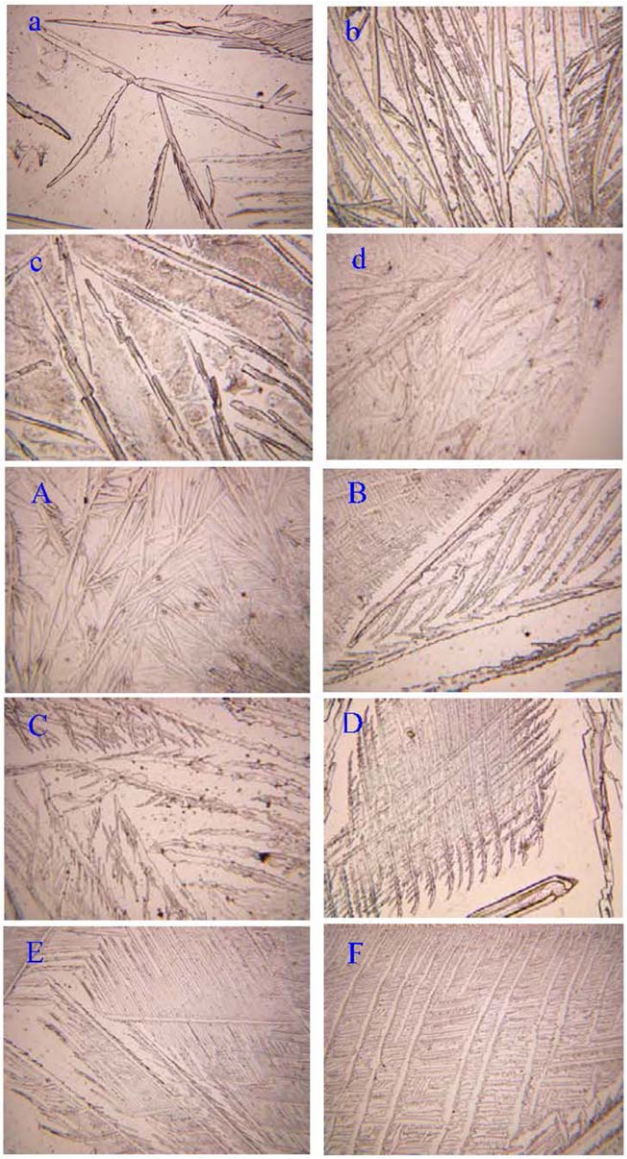

Figure 3. Microphotographs of (a–d) sweet potato amylose and (A–D) amylopectin during repeated retrogradation (from first to fourth time). (E) Amylose bound together during first retrogradation; (F) amylopectin bound together during first retrogradation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

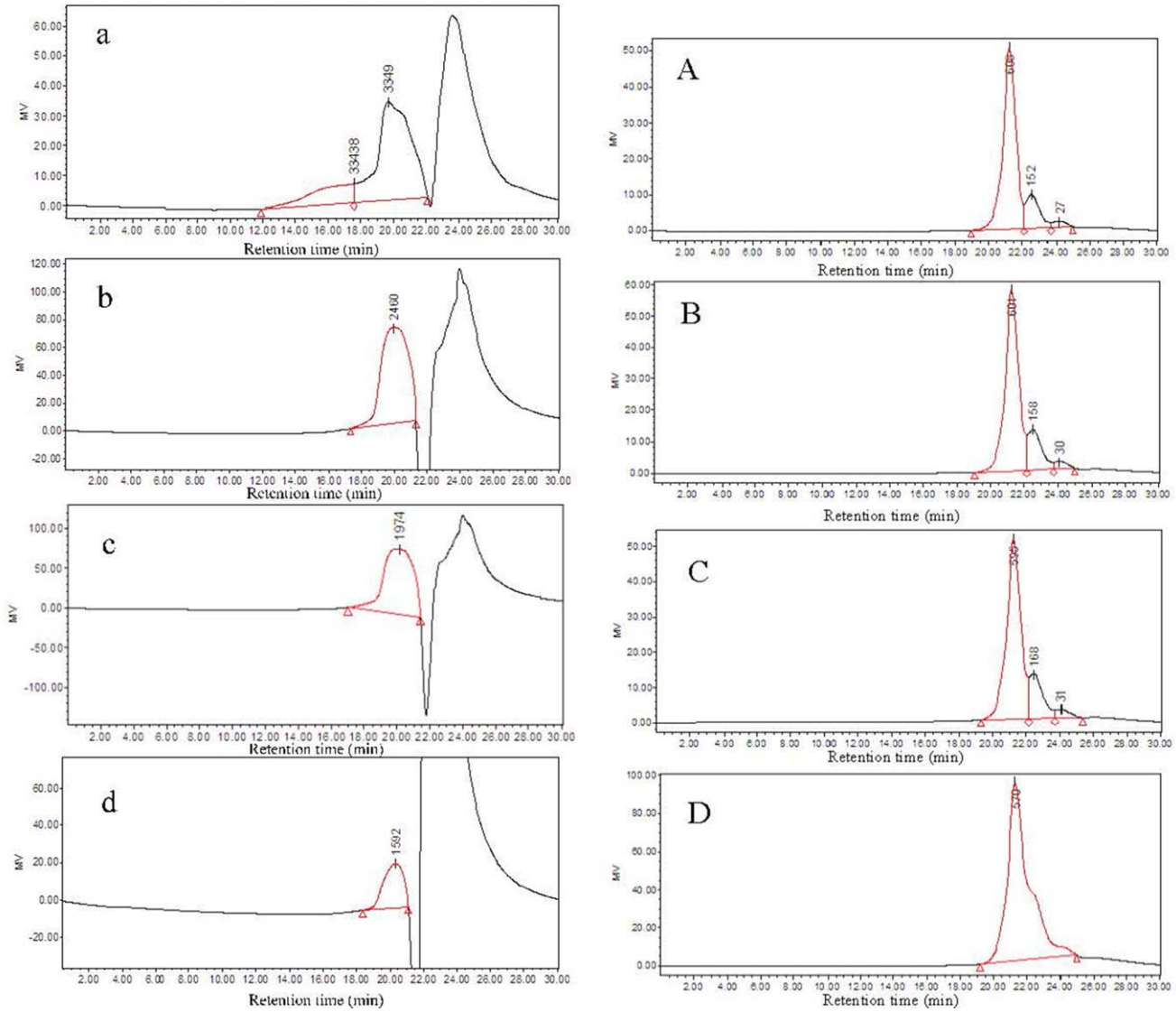

Figure 4. Molar mass distribution of retrograded sweet potato starches: (a–d) sweet potato amylopectins retrograded for one, two, three, and four times, respectively; (A–D) sweet potato amyloses retrograded for one, two, three, and four times, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

involved in crystallization will be hydrolyzed in the purification of retrograded starches. When the lengths of the branching chains are reduced to their limits (for amylose DP 3–13), the crystals in Figure 3(c,D) will be present. In other words, the crystal of a narrow chain length of starch contains more hydrogen bonds. Compared with starch not involved in

Table I. Effect of Repeated Retrogradation on Molar Mass of Sweet Potato Starch

		Sequence of retrogradation			
		1	2	3	4
Amylose	MW (g mol^{-1})	3349 ± 20	2460 ± 13	1974 ± 30	1592 ± 20
	Distribution of MW (g mol^{-1})	$4.2 \times 10^7 \pm 18$ to 205 ± 6	$1.0 \times 10^5 \pm 13$ to 554 ± 3	$6.7 \times 10^4 \pm 12$ to 534 ± 4	$6.0 \times 10^4 \pm 11$ to 730 ± 5
Amylopection	MW (g mol^{-1})	595 ± 4	608 ± 2	601 ± 2	570 ± 3
	Distribution of MW (g mol^{-1})	7971 ± 5 to 223 ± 3	6681 ± 6 to 215 ± 2	5408 ± 7 to 183 ± 5	4533 ± 9 to 211 ± 1

The results are expressed as the mean ± standard deviation of triplicate measurements.

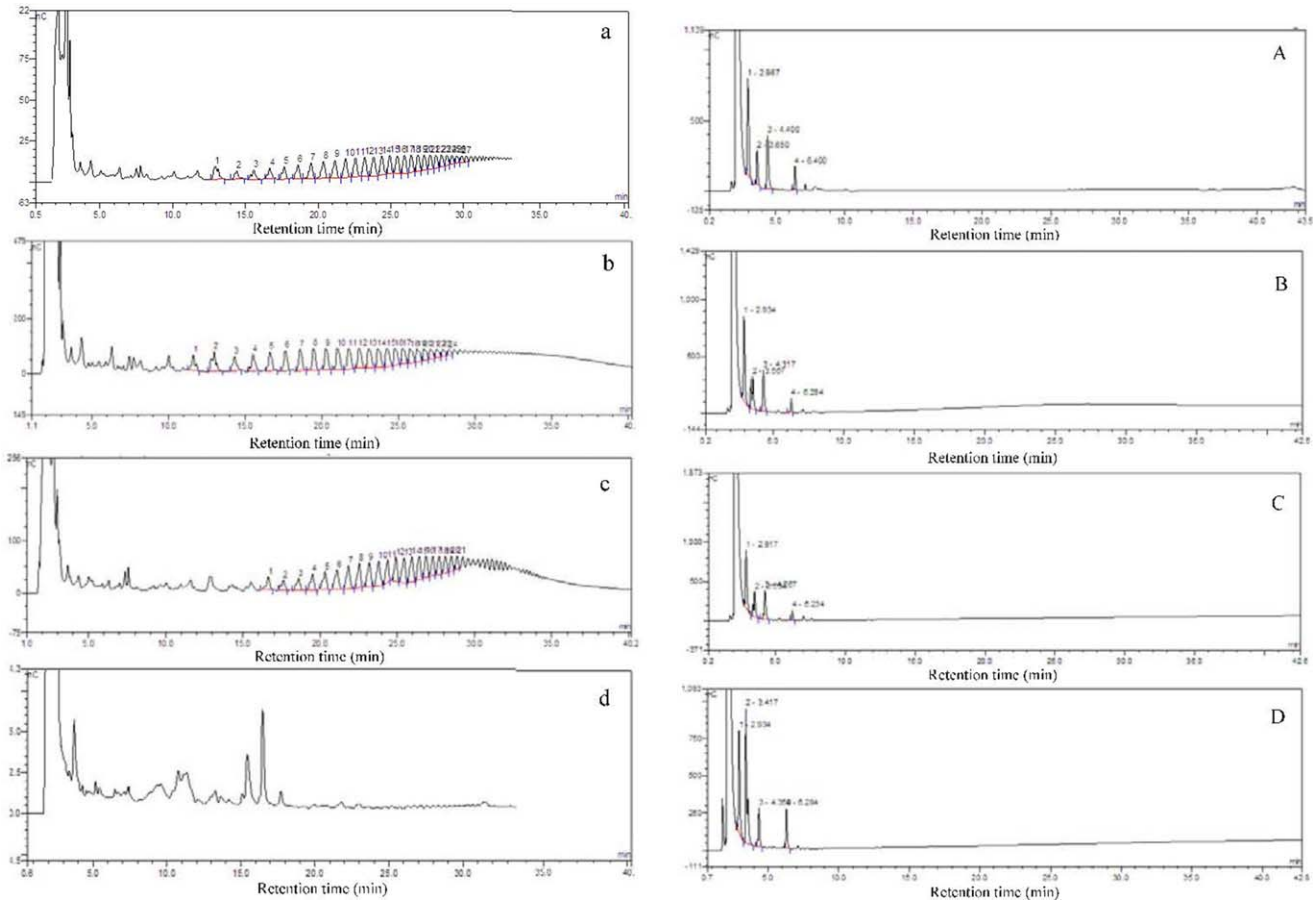

Figure 5. Chain length distribution of retrograded sweet potato starches: (a–d) sweet potato amylopectins retrograded for one, two, three, and four times, respectively; (A–D) sweet potato amyloses retrograded for one, two, three, and four times, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

retrogradation in the literature,[36] the branching chain in retrograded sweet potato amylose and amylopectin stretches forth more straightly.

Molar Mass Analysis

The molar mass distributions of sweet potato amylose and amylopectin retrograded repeatedly are presented in Figure 4 and Table I.

There are two obvious changes in the molar mass during repeated retrogradation. One is the narrower distribution of molar mass (Table I), and in the other, only one peak is left after the third retrogradation–hydrolysis (Figure 4). The data in Table I show that repeated retrogradation–hydrolysis from one to three times causes the molar mass distribution of sweet potato amylose to reduce from 4.20×10^7–205 to 1.5×10^5–554, 6.7×10^4–534, and 6.0×10^4–730 g mol^{-1}, respectively, which correspond to DP values of 1–259259, 3–925, 3–413, and 4–370. For sweet potato amylopectin, they reduced from 7971–223 to 6681–251, 5408–183, and 4533–211 g mol^{-1} respectively, corresponding to DP 1–49, 1–41, 1–33, and 1–27. The largest molar mass of sweet potato amylose in retrograded starch is equal to that of native starch, but that of amylopectin is much less than that of native starch.[37] This indicates that hydrogen bonds might form between sweet potato amyloses with long chain lengths, but not between amylopectins with long chain length, which might be because of hindrance of its branched chains. The molar masses of 205, 223, 730, and 211 g mol^{-1} in Table I are ascribed to a single carbohydrate or polysaccharide combined with different ions, as follows: 205 (glucose + $Na^+ + 2H^+$), 223 (glucose + $K^+ + 4H^+$), 730 (4 glucose + $K^+ + Na^+ + 2H^+$), and 211 (glucose + $Na^+ + 8H^+$). The weight-average molar mass (MW) of sweet potato amylose and amylopectin during the first to fourth retrogradation–hydrolysis in Table I reduces from 3349 to 1592 g mol^{-1} and from ~600 to 570 g mol^{-1}, respectively. These molar masses involved in retrogradation are much lower than that of native sweet potato starch.[9] When the MW of amylopectin reduces below 516 g mol^{-1}, the formation of double helices is hindered, as shown in Figure 1(D).

Chain Length Analysis

Small changes in the molecular structure of amylopectin give rise to sharp changes in the retrogradation ratio, showing that the molecular structure is important for the crystallization of amylopectin.[38] Figure 5 and Table II show the polymer chain distributions of the sweet potato amylose and amylopectin after repeated retrogradation.

Table II. Effects of Repeated Retrogradation on Chain Length Distribution of Sweet Potato Starch

	Sequence of retrogradation							
	1		2		3		4	
	DP	Percentage	DP	Percentage	DP	Percentage	DP	Percentage
Amylose	9	5.05 ± 0.04	8	3.66 ± 0.05	12	3.16 ± 0.13[a]	3	27.43 ± 0.04
	10	2.45 ± 0.07	10	5.8 ± 0.11[a]	13	2.71 ± 0.16[a]	11	22.38 ± 0.12[a]
	11	3.15 ± 0.14	11	3.85 ± 0.02[b]	14	3.3 ± 0.03[b]	12	40.86 ± 0.03[b]
	12	2.82 ± 0.01	12	4.3 ± 0.13[a]	15	4.21 ± 0.12[a]	13	9.33 ± 0.07[b]
	13	3.52 ± 0.12	13	4.72 ± 0.02[b]	16	4.8 ± 0.06[b]		
	14	3.78 ± 0.05	14	4.88 ± 0.15[a]	17	5.74 ± 0.15[a]		
	15	4.21 ± 0.03	15	5.23 ± 0.06[b]	18	6.24 ± 0.07[b]		
	16	4.64 ± 0.12	16	5.3 ± 0.13[a]	19	5.97 ± 0.14[a]		
	17	5.01 ± 0.06	17	5.06 ± 0.17[a]	20	6.33 ± 0.03[b]		
	18	5.12 ± 0.08	18	5.82 ± 0.08[b]	21	6.74 ± 0.04[b]		
	19	4.85 ± 0.14	19	5.59 ± 0.11[a]	22	5.22 ± 0.02[b]		
	20	4.86 ± 0.11	20	5.26 ± 0.09[a]	23	5.22 ± 0.12[a]		
	21	5.08 ± 0.10	21	5.32 ± 0.04[b]	24	6.55 ± 0.17[a]		
	22	4.78 ± 0.21	22	5.25 ± 0.18[a]	25	6.56 ± 0.13[a]		
	23	4.19 ± 0.16	23	5.07 ± 0.06[b]	26	5.5 ± 0.16[a]		
	24	4.38 ± 0.09	24	4.24 ± 0.12[a]	27	4.62 ± 0.02[b]		
	25	4.58 ± 0.03	25	3.85 ± 0.01[b]	28	4.2 ± 0.17[a]		
	26	4.15 ± 0.04	26	3.76 ± 0.13[a]	29	4 ± 0.05[b]		
	27	3.8 ± 0.15	27	3.31 ± 0.18[a]	30	3.7 ± 0.11[a]		
	28	3.48 ± 0.12	28	2.79 ± 0.05[b]	31	2.98 ± 0.03[b]		
	29	3.2 ± 0.05	29	2.31 ± 0.16[a]	32	2.24 ± 0.18[a]		
	30	2.94 ± 0.14	30	2 ± 0.14[a]				
	31	2.52 ± 0.03	31	1.55 ± 0.09[a]				
	32	2.1 ± 0.09	32	1.07 ± 0.02[b]				
	33	1.69 ± 0.01						
	34	1.36 ± 0.07						
	35	2.27 ± 0.02						
Amylopectin	2	45.74 ± 0.05	2	45.56 ± 0.06[a]	2	46.87 ± 0.11[a]	2	32.5 ± 0.06[b]
	3	14.2 ± 0.11	3	24.93 ± 0.15[a]	3	23.04 ± 0.04[b]	3	44.42 ± 0.03[b]
	4	30.89 ± 0.13	4	23.85 ± 0.12[a]	4	24.56 ± 0.15[a]	4	12.18 ± 0.08[b]
	5	9.18 ± 0.06	5	5.66 ± 0.13[a]	5	5.52 ± 0.13[a]	5	10.9 ± 0.17[a]

The results are expressed as the mean ± standard deviation of triplicate measurements. Values with superscripts in the same row were significantly different (a: $P < 0.05$; b: $P < 0.01$).

The ranges of chain length distributions of sweet potato amylose for the first to the fourth retrogradation–hydrolysis are DP 9–35, 8–32, 12–32, and 3–13, respectively; but those for sweet potato amylopectin are all DP 2–5. Such results hint that sweet potato amylose has a wide range of chain length distributions, and only the A chain of sweet potato amylopectin is involved in retrogradation. Those A chains have narrow ranges of chain length distributions. Other workers have reported DP values of 20–30,[20] 19–26,[39] and 13–17[40] in resistant starch isolated from recrystallized starches of different origins. So we now know that those DP values only represent amylose but not amylopectin. For normal starch containing amylose and amylopectin, it is impossible for amylopectin not to be involved in retrogradation. The detailed chain length distributions of sweet potato starch during repeated retrogradation are presented in Table II. Compared to the results of visible absorbance in Figure 1(D), this suggests that the double helix will not form if the percentage of chain lengths with DP ≥ 4 is less than 25%. The results of Figure 1 and Figure 5 also imply that there is no linear relationship between the visible absorbance wavelength of the starch–iodine complex and its chain length. Most short double helices in amylopectin might go together to form a higher visible absorbance wavelength of the starch–iodine complex. The results in Figure 5 also explain why not all waxy starches display low retrogradation ratios,[41] and the contents of A chains with DP 2–5 in amylopectin of those starches are probably very high and give rise to a high nucleation of retrogradation.

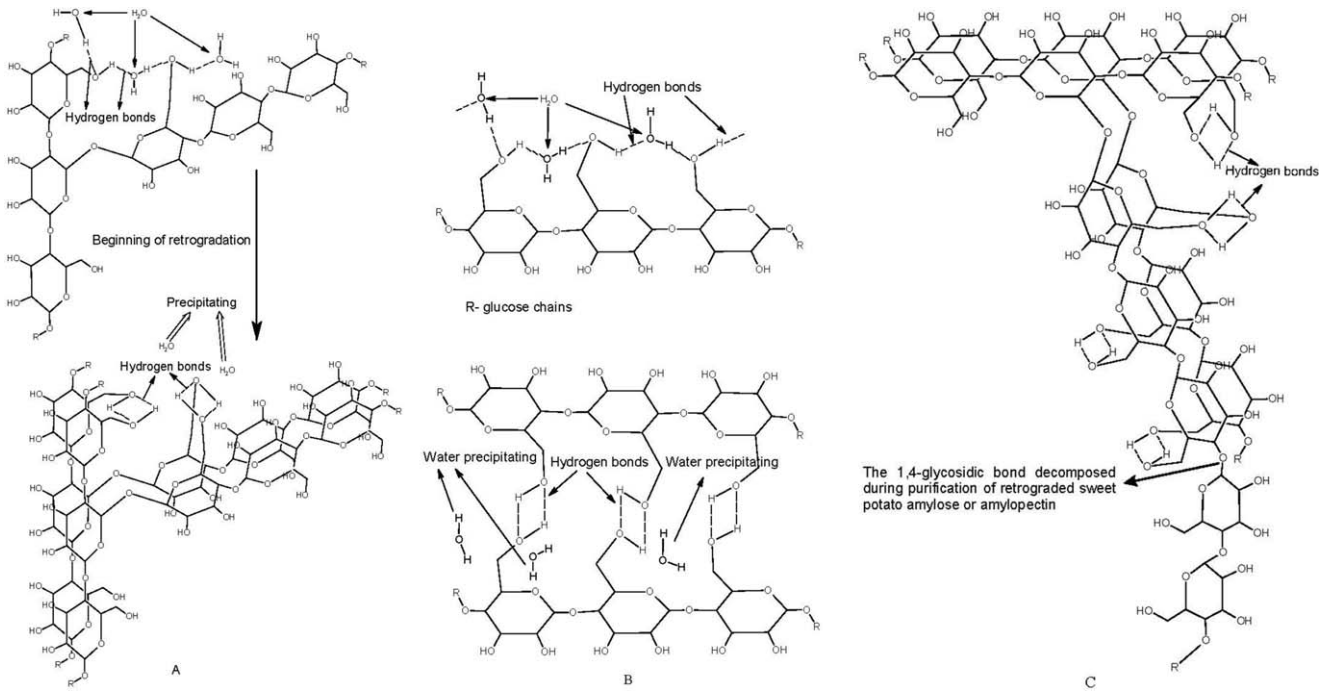

Figure 6. Schematic diagrams of the possible way to obtain sweet potato amylose and amylopectin with narrow molar mass distribution by repeated retrogradation: (A) beginning of retrogradation; (B) forming of retrograded chains; (C) purification of retrograded starch.

Possible Way to Obtain Narrow Molar Mass Distribution of Sweet Potato Amylose and Amylopectin by Repeated Retrogradation

Based on the current results, a schematic diagram of the mechanism of chemical changes for sweet potato amylose and amylopectin during repeated retrogradation is presented in Figure 6. First, the starch is resolved in water during gelatinization to form three kinds of hydrogen bonds between the sixth carbon atoms of adjacent glucose units linked by the α-1,6-glycosidic bond or by the α-1,4-glycosidic bond [Figure 6(A,B)], or between the sixth carbon atoms from different chains and water. Those weak hydrogen bonds offer mobility to the starch and hinder the aggregation of starch chains. The retrogradation begins as the temperature of starch paste decreases. At this moment, the hydrogen bonds between water and the sixth carbon atoms at the α-1,6-glycosidic bond in Figure 6(A) are first broken, and a new hydrogen bond forms between those two carbon atoms. The water originally involved in the hydrogen bond is forced out of the starch solution in the course of new hydrogen bond formation. What is very important is that this course might be the nucleation of retrogradation crystals and lead to the presence of the identical picture in Lian *et al.*[15] Then a great number of hydrogen bonds form between two chains linked by the α-1,4-glycosidic bond, as shown in Figure 6(B). Lots of water is separated out from the starch solution, which usually happens during the short-term retrogradation. For sweet potato amylopectin, not all hydroxyls form hydrogen bonds with water in the course of starch pasting because of its cluster structure. So during the long-term retrogradation, the water crushes in amylopectin to form hydrogen bonds with the sixth carbon atom of amylopectin and breaks the cluster. Then the water moves from here to there, and the same retrogradation course happens. The regular arrangement of amylopectin, formed by retrogradation, further opens the interval of different clusters, and more water goes into clusters, as shown in Figure 2. The narrower distribution of molar mass and chain length during repeated retrogradation is interpreted in Figure 6(C). When long and short chains form hydrogen bonds, part of the long chain will not be involved in hydrogen bond formation and will be decomposed by α-amylase during the purification of retrograded starch.

CONCLUSIONS

Repeated retrogradation–hydrolysis is a potential method to obtain amylose or amylopectin with a narrow distribution of molar mass or chain length. The mechanism for the procedure is as follows. First the hydrogen bonds form between starch and water, then the water is replaced by chains with different lengths, and finally the long chains linked with short ones by hydrogen bonds will be cut at the position where no hydrogen bonds form. Such starches might serve as ideal materials to prepare crystals of retrograded starch with sharp X-ray diffraction peaks, which offers us a chance to know more about retrogradation.

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