

Structure–Retrogradation Relationship of Rice Starch in Purified Starches and Cooked Rice Grains: A Statistical Investigation

YUAN YAO,* JINGMIN ZHANG,[†] AND XIAOLIN DING

Department of Food Science, Wuxi University of Light Industry, Wuxi, Jiangsu, 214036, P.R. China

Amylose content and amylopectin chain length distribution, the two most commonly used structural parameters of starch, have significant effects on starch retrogradation. In the present work, starches were separated and purified from 18 rice cultivars. The amylopectin was purified from each starch. Amylopectin chain length distribution was analyzed by high-performance size-exclusion chromatography after debranched using isoamylase. The blue value was used to measure the amylose content before and after the defatting of starch. The amount of amylose associated with lipid was calculated. Pulsed nuclear magnetic resonance was used to follow the retrogradation of starch both in cooked rice grains and in the purified form. The Avrami equation was employed to describe the retrogradation kinetics of rice starch. To look into the relationship between the starch structure and retrogradation behavior, the structural parameters were correlated with retrogradation kinetics parameters using both Pearson and partial correlations. The results indicated the following: first, the retrogradation behavior of rice starch remains similar in both the purified form and cooked rice grains; second, the peak value of amylopectin short-chain length has a significant positive relationship with the amylopectin crystallization rate constant k ; third, the amylose content after defatting has a significant positive relationship with the parameter k and a negative relationship with the Avrami exponent n ; and fourth, the amount of amylose associated with lipid has a negative relationship with the parameter k .

KEYWORDS: Rice starches; amylopectin structure; amylose content; lipid; retrogradation kinetics; PNMR

INTRODUCTION

Understanding the structure–function relationship of starch is of fundamental importance in both starch application and the engineering of starch structure by genetic methods and post-harvest processing. Starch is composed of two types of macromolecules, amylose and amylopectin. Amylose is essentially a linear α -1,4-linked glucan chain of approximately 1000 residues, whereas amylopectin has numerous branch points that form by α -1,6 linkages joining linear chains, and it has a degree of polymerization (DP) up to tens of thousands. After gelatinization, the external chains of amylopectin have the tendency to form double helices that will further aggregate into crystallites. The recrystallization process of starch, named retrogradation, is the major reason for the deteriorated qualities of cereal food during storage. Understanding the relationship between starch structure and retrogradation is essential for producing cereal food with extended shelf life. Factors affecting starch retrogradation include the structure of amylopectin (1–6), the content of amylose (7–13), and the existence of non-

starch components such as protein (14, 15) and lipids (16–20). Most research has focused on the relationships between retrogradation-related physical properties and certain chemical properties of starch systems. Due to the difficulty in preparing model starch systems with multiple chemical variables, most often the data were simply compared; thus, little had been addressed on the effect of the interactions among various starch chemical parameters. Hence, some researchers started to investigate the effects of multiple chemical variables on retrogradation by statistical methods (6). But a comprehensive study on the relationship between starch chemical structure and retrogradation kinetics has not been reported yet.

Pulsed nuclear magnetic resonance (PNMR) has been used for studies on the retrogradation of starch and cereal foods (22–27). In contrast to other methods, PNMR has been shown to be rapid, reproducible, and nondestructive, which is important in monitoring the retrogradation process of regular cereal food products. Thus, we employed PNMR to kinetically monitor the retrogradation process of rice starches.

The Avrami equation has been used to describe polymer crystallization kinetics during an isothermal process (28). This equation has been applied in the research on starch retrogradation for characterizing the crystallite formation in gelatinized starch systems (29–34). In this paper, the parameters in the

* Corresponding author. Current address: 115 Borland Laboratory, Department of Food Science, The Pennsylvania State University, University Park, PA 16802. Phone: (814) 863-7958. Fax: (814) 863-6132. E-mail: yoy1@psu.edu.

[†] Current address: 302 Wartik Laboratory, The Pennsylvania State University, University Park, PA 16802.

Avrami equation were used to describe the starch retrogradation kinetics.

In the present work, the structural parameters of starches from 18 rice cultivars were correlated with Avrami parameters describing the retrogradation of starches both in the purified form and in cooked rice grains. Using this approach, we tried to provide more information on the relationship between starch chemical structure and retrogradation behavior.

MATERIALS AND METHODS

Milled Rice, Rice Starch, and Rice Amylopectin. The milled rice from different cultivars was collected and kindly provided by the Chinese Institute of Agriculture (Beijing, China). Most cultivars are of commercial importance. The major morphological difference between the grains of two types of waxy rice (93-124 waxy rice and common waxy rice) is that the common waxy rice is a medium-grain rice, whereas the 93-124 is a long-grain rice. The crops for all the samples were harvested and processed in 1998.

Rice flour suspensions were prepared from milled rice soaked in 5 equiv (w/w) of deionized water using an electric homogenizer until the suspension passed through a 100-mesh sieve. After centrifugation, the precipitate was dried at 40 °C until the moisture content was lower than 8%. For rice starch, rice flour was soaked in 5 equiv (w/w) of 0.4% NaOH solution at room temperature for 48 h and washed with deionized water repeatedly until the pH reached 7. The starch precipitate was dried at 40 °C until the moisture content was lower than 6%. Amylopectin from rice starch was prepared according to the method of Takeda et al. (35, 36). For the measurement of moisture content, a sample was first dried at 40 °C in an oven for 48 h to remove most of the water, and then it was heated at 105 °C to constant weight when weighed at an interval of 12 h.

Amylose Content before and after Defatting. The defatting of starch was performed according to Takeda et al. (35). The blue value of the starch dispersion was used to quantify the amylose content. The measurement was performed as follows. One hundred milligrams of starch sample was dissolved in 10.0 mL of 90% DMSO and then diluted to 50.0 mL with deionized water. An aliquot of 2.0 mL was diluted with deionized water to 50.0 mL, and then 1.0 mL of iodine reagent (0.30% I₂ + 3.0% KI) was added. After vortexing, the absorbance at 600 nm was measured. The standard curve was prepared using mixtures of amylopectin isolated from Huwan rice starch and amylose from potato starch (Sigma, St. Louis, MO).

The amylose content of rice starch was described using three parameters: amylose content before defatting (AM), amylose content after defatting (AM₀), and amylose content associated with lipid (ΔAM). ΔAM is the difference between AM₀ and AM (ΔAM = AM₀ - AM).

Amylopectin Chain Length Distribution. The debranching of amylopectin was carried out as follows. Amylopectin (50 mg) was dissolved in sodium acetate buffer (5 mL, 0.05 M, pH 3.65). Isoamylase (20 μL, 10 000 units, Sigma) was then added, followed by incubation at 25 °C for 24 h. After incubation, the solution was heated in boiling water for 5 min to inactivate the enzyme.

The amylopectin chain length distribution was determined by high-performance size-exclusion chromatography (HPSEC). The HPSEC system comprised a Rheodyne model 7725 sample injector, a Waters 510 pump, a Waters 2410 refractive index detector, a Water 740 data module, and three columns (TSK gel G2000SWXL, Shodex KW803, and TSK gel G4000 SW) connected in series. The mobile phase was sodium acetate buffer (0.05 M, pH 3.65) with a flow rate of 0.8 mL/min. The standards used to calibrate the column system included amylose (DP18) (Hayashibara Inc., Okayama, Japan), maltotriose and maltoheptaose (Sigma), and dextrans (*M_w* 41 000 and 580 000, Sigma).

The chromatogram of amylopectin chain length distribution was described using three parameters: DP₁, the chain length (in degree of polymerization, DP) at the climax of the left peak (peak 1), corresponding to long chains; DP₂, the chain length at the climax of the right peak (peak 2), corresponding to short chains; and P₁/P₂, the ratio between the amounts of long chains and short chains.

PNMR Used To Monitor the Starch Retrogradation. The retrogradation behaviors were analyzed by PNMR (20 MHz, Minispec PC120, Bruker) equipped with a 10 RTS probe. The size of the test tube was 180 mm × 10 mm. The EDM 110A was used to measure the relative solid content (*S'*, %) of each sample. Conditions were magnetic temperature 40 °C, signal attenuation 38, offset 0.135, 90° pulse width of 1.14 μs; each sample was scanned nine times. The registered times were taken at 11 and 59 μs. The calibration factor was 1.475, determined by standards with predetermined relative solid content values.

Cooked Rice Grains and Rice Starches Used for Retrogradation Analysis. For the cooked rice grains, the milled rice with known moisture content (about 14.5%) and deionized water were added into the PNMR tubes in ratio of about 1:1.2 to make the moisture content 61.1%. The tubes were sealed and the samples heated by steam under normal pressure for 30 min. After heating, the samples were stabilized at 4 °C for 30 min. *S'*₀ (initial *S'* of retrogradation monitoring) was then measured using PNMR. The *S'* values during the 4 °C storage were also measured after 1, 2, 3, 4, 5, 7, 9, 11, 12, 14, 16, and 18 days. All samples were prepared in duplicate, and the mean values were used for analysis.

For the preparation of rice starch samples, the starches (moisture content about 6%) were mixed with deionized water to give suspensions with a moisture content of 70.0%. After vortexing, the suspensions were added into the PNMR tubes, the tubes were sealed, and the samples were heated in a boiling water bath for 30 min. After heating, the samples were stabilized at 4 °C for 30 min. The *S'*₀ was then measured using PNMR. The *S'* values during the 4 °C storage were also measured after 1, 2, 3, 4, 5, 6, 8, 10, 12, 13, 15, and 18 days. All samples were prepared in duplicate, and the mean values were used for analysis.

Avrami Equation. In the Avrami equation,

$$V = 1 - \exp(-kt^n)$$

V is defined as the crystallite percentage of the limiting value at storage time *t*. The parameter *k* is the crystallization rate constant, and in theory it is related to the number of nuclei and the crystallite growth rate. The parameter *n* is the Avrami exponent and relates to the crystallite growth mode. Both *k* and *n* can be obtained by linear regression of the equation

$$\ln(-\ln(1 - V)) = \ln k + n \ln t$$

derived from the original Avrami equation. When *S'* is the relative solid content measured by PNMR, *V* can be calculated as

$$V = (S'_t - S'_0)/(S'_{\text{Retro}} - S'_0)$$

*S'*₀ is the initial *S'* of retrogradation monitoring. *S'*_{Retro} is the *S'* at the end of the storage period. *S'*_{*t*} is the *S'* at storage time *t*.

Avrami parameters used to describe the retrogradation kinetics are *n*₁ and *k*₁ for cooked rice grains, and *n*₂ and *k*₂ for purified rice starches.

Statistical Analysis. Pearson and partial correlations were carried out using SAS Software version 8.1 (SAS Institute Inc., Cary, NC).

RESULTS

Amylopectin Structure. The chain length distributions of amylopectin in two waxy starches (93-124 and common waxy), one low amylose starch (Jiran), one normal starch (Jingyu 13), and one high amylose starch are shown in **Figure 1**. For each rice starch, the chain length at the climax of peak 1, corresponding to long chains (DP₁), the chain length at the climax of peak 2, corresponding to short chains (DP₂), and the ratio between long and short chains (P₁/P₂) are shown in **Table 1**. The amylopectin structure of 93-124 starch is distinctive because of the much wider length distribution of long chains compared to those in other starches. The amylopectin chain length distributions of other starches are not as distinctive as for

Table 1. Retrogradation Kinetics and Structural Parameters of Rice Starches in the Purified Form and in Cooked Rice Grains

| sample | n_1^a | k_1^b | n_2^a | k_2^b | DP ₁ ^c (DP) | DP ₂ ^c (DP) | P ₁ /P ₂ ^d | AM ^e (%) | AM ₀ ^e (%) | ΔAM ^f (%) |
|--------------|---------|---------|---------|---------|--------------------------------------|--------------------------------------|---|------------------------|-------------------------------------|-------------------------|
| 93-124 | 1.565 | 0.043 | 1.474 | 0.027 | 60.8 | 15.4 | 0.479 | 0.0 ± 1.1 | 0.0 ± 0.9 | 0.0 |
| Dalian | 0.964 | 0.278 | 0.866 | 0.242 | 40.2 | 15.7 | 0.217 | 22.1 ± 1.6 | 24.8 ± 1.4 | 2.7 |
| Dongfan | 0.966 | 0.304 | 0.991 | 0.194 | 38.6 | 15.5 | 0.224 | 26.2 ± 0.8 | 27.0 ± 1.0 | 0.8 |
| H 783 | 0.844 | 0.411 | 0.864 | 0.262 | 40.0 | 15.4 | 0.310 | 22.9 ± 1.1 | 27.7 ± 0.6 | 4.8 |
| high amylose | 0.551 | 0.970 | 0.565 | 0.868 | 40.8 | 18.2 | 0.243 | 30.0 ± 1.9 | 35.2 ± 1.7 | 5.2 |
| Huwan | 1.110 | 0.187 | 1.235 | 0.096 | 39.2 | 15.7 | 0.222 | 19.5 ± 1.3 | 26.2 ± 1.3 | 6.7 |
| Jingyu 13 | 1.062 | 0.216 | 1.145 | 0.108 | 41.2 | 16.0 | 0.242 | 15.8 ± 0.7 | 24.0 ± 0.9 | 8.2 |
| Jingyu 21 | 1.156 | 0.135 | 1.140 | 0.101 | 39.3 | 15.4 | 0.265 | 14.3 ± 0.9 | 22.7 ± 1.1 | 8.4 |
| Jiran | 1.436 | 0.079 | 1.450 | 0.034 | 40.5 | 15.4 | 0.266 | 11.3 ± 0.8 | 14.3 ± 0.9 | 3.0 |
| Liao 135 | 0.982 | 0.300 | 0.969 | 0.182 | 41.1 | 16.7 | 0.278 | 18.8 ± 1.0 | 25.9 ± 0.9 | 7.1 |
| Qiyuyu 62 | 1.029 | 0.268 | 1.078 | 0.131 | 41.6 | 16.3 | 0.248 | 18.4 ± 1.1 | 26.2 ± 1.3 | 7.8 |
| R 1017 | 0.909 | 0.311 | 1.029 | 0.168 | 39.4 | 16.6 | 0.230 | 20.6 ± 1.0 | 30.7 ± 1.2 | 10.1 |
| Tian 305 | 0.936 | 0.283 | 1.067 | 0.141 | 40.4 | 15.5 | 0.261 | 19.5 ± 0.8 | 25.1 ± 1.1 | 5.6 |
| common waxy | 1.308 | 0.112 | 1.761 | 0.026 | 40.4 | 16.2 | 0.368 | 0.0 ± 1.2 | 0.0 ± 1.0 | 0.0 |
| Xiang 228 | 0.940 | 0.273 | 1.323 | 0.07 | 39.2 | 15.3 | 0.229 | 19.9 ± 0.8 | 26.5 ± 1.2 | 6.6 |
| Yi 947 | 0.905 | 0.306 | 1.116 | 0.128 | 41.4 | 15.5 | 0.258 | 17.3 ± 0.9 | 25.1 ± 1.1 | 7.8 |
| Yuefu | 0.970 | 0.284 | 1.029 | 0.159 | 40.3 | 15.9 | 0.252 | 22.1 ± 1.1 | 27.0 ± 1.3 | 4.9 |
| Zhongzuo 93 | 0.927 | 0.325 | 1.227 | 0.099 | 40.4 | 15.2 | 0.222 | 18.4 ± 1.0 | 25.5 ± 1.1 | 7.1 |

^a Avrami parameter related to crystallite growth mode of amylopectin in cooked rice grains, n_1 , and purified rice starch, n_2 . ^b Avrami parameter describing the crystallization rate constant of amylopectin in cooked rice grains, k_1 , and purified rice starch, k_2 . ^c Chain length (degree of polymerization, DP) at the climax of peak 1, corresponding to long chains, DP₁, and at the climax of peak 2, corresponding to short chains, DP₂. ^d Ratio between the amount of long chains and short chains. ^e Amylose content before defatting, AM, and after defatting, AM₀. The data are expressed as mean ± SD. ^f Content of amylose associated with lipid.

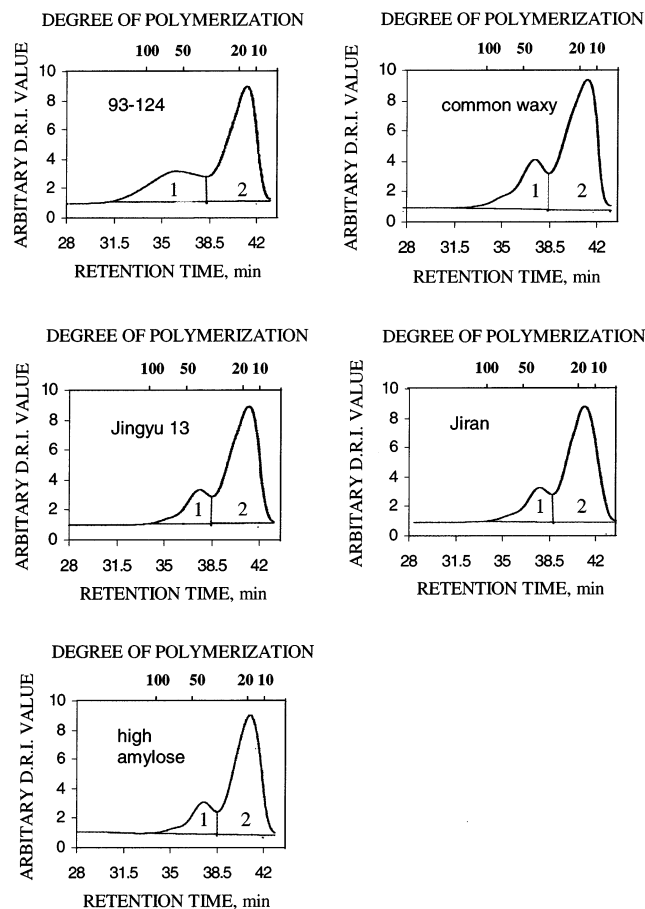


Figure 1. High-performance size-exclusion chromatography (HPSEC) of debranched amylopectin dispersions from five rice cultivars. The chromatogram profile of each amylopectin can be separated into two peaks, peak 1 and peak 2. Parameters describing the chain length distribution include the chain length at the climax of peak 1, corresponding to long chains, DP₁; the chain length at the climax of peak 2, corresponding to short chains, DP₂; and the ratio between the amounts of long chains and short chains, P₁/P₂.

93-124 starch, yet the differences between the starches can still be identified in Table 1. DP₁ and DP₂ are not necessarily

integrals since they are statistically averaged values calculated from the standard curve. Generally, DP₁ ranges from 38.6 to 41.6, with one exception for 93-124 (DP₁ 60.8). DP₂ ranges from 15.2 to 18.2, with that of high amylose rice starch being the highest. P₁/P₂ ranges from 0.217 to 0.479. The two waxy rice starches have much higher P₁/P₂ than the others (93-124, 0.479; common waxy, 0.368).

Amylose Content. The amylose contents of 18 rice starches before and after defatting are shown in Table 1. Since the long external chains of amylopectin may contribute to the blue value, what is shown is the apparent amylose content. Yet the contribution of amylopectin long external chains to the apparent amylose content is determined by the standards used in the blue value measurement. In the current study, the standard for 0% amylose content was the amylopectin isolated from Huwan rice starch, which could remove the effect of amylopectin long external chains on the amylose content determinations for all starch samples.

The extra-long chains in peak 1 (Figure 1) of the two waxy starches constitute a portion of amylopectin internal chains and have no detectable effects on the amylose contents. Thus, both AM and AM₀ of two waxy starches were undetectable.

For all nonwaxy starches, the amylose content before defatting (AM) is lower than that after defatting (AM₀). AM and AM₀ of high amylose rice starch are 30 and 35.2. Amylose contents of other rice starches range from 11.3 to 26.2 for AM and from 14.3 to 30.7 for AM₀.

Content of Amylose Associated with Lipid. A portion of amylose may form helical complexes with internal lipid in starch granules and cannot associate with iodine. Thus, the amylose content indicated by the blue value is affected by the amount of amylose associated with lipid. Keetels et al. (20) reported the formation of complexes between amylose and lipid surfactants, and that no effect of lipid on the mechanical properties of waxy maize starch (amylopectin) was observed. Thus, we considered that the difference between AM and AM₀ (ΔAM) originates, mostly if not exclusively, from the amylose–lipid complex and can be used as a proper and convenient index for quantifying the amylose associated with lipid. The value of ΔAM ranges from 0.0% for waxy rice starches to 10.1% for

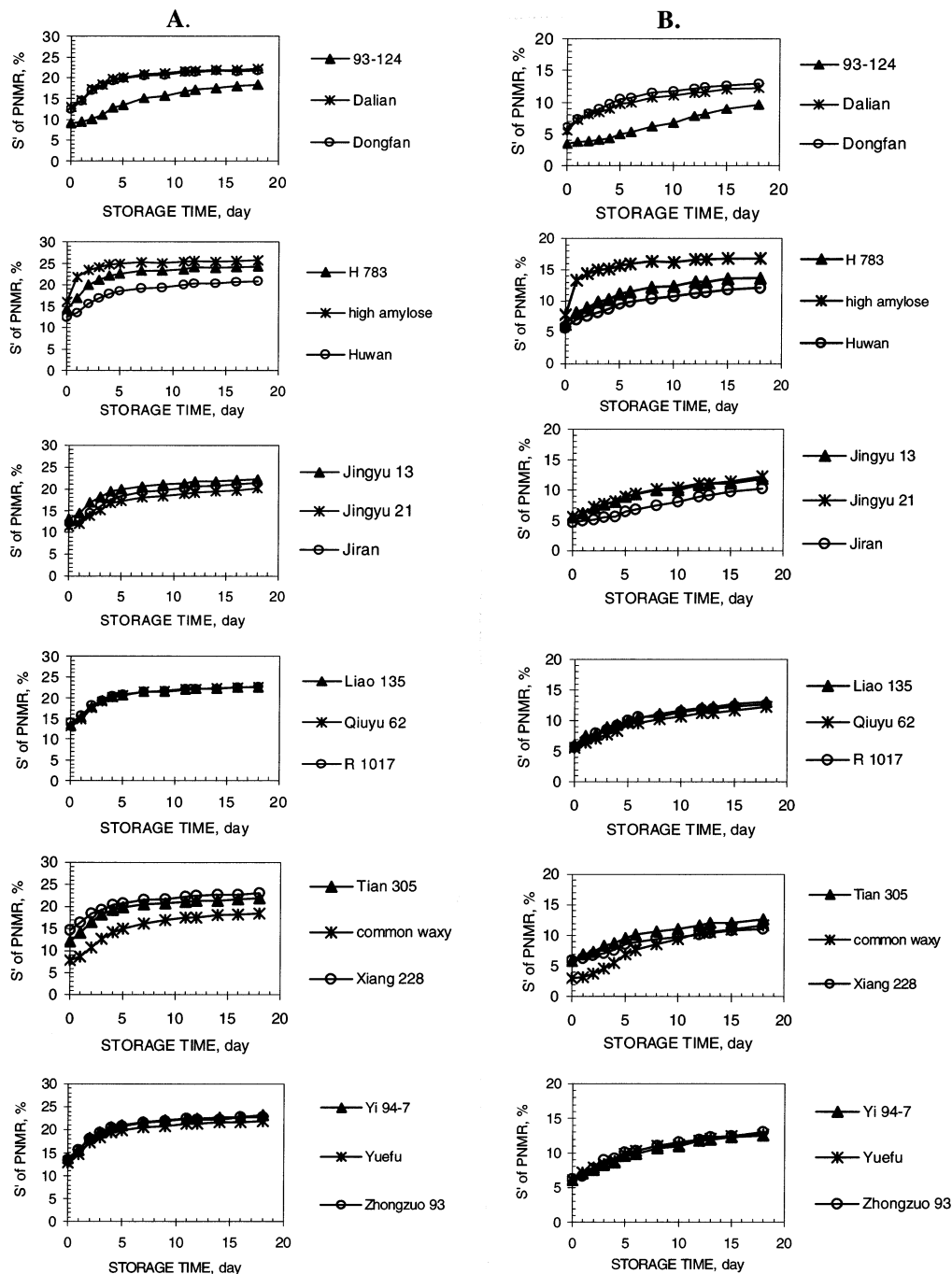


Figure 2. Starch retrogradation in cooked rice grains (column A) and purified rice starches (column B) monitored by pulsed nuclear magnetic resonance (PNMR). The X-axis shows the storage time at 4 °C. The Y-axis shows the relative solid content, S' , measured by PNMR.

R 1017 rice starch. It is interesting to note that ΔAM is not necessarily related to AM_0 . The high amylose rice starch with an AM_0 of 35.2% has a ΔAM of only 5.2%.

Retrogradation Behavior of Rice Starches. Figure 2 shows the retrogradation behavior of rice starches in cooked rice grains and in the purified form. For the convenience of comparison, the sample orders of columns A and B are the same. In column B, it is clearly shown that for the whole storage period, two waxy rice starches, 93-124 and common waxy rice starch, had the lowest S' values compared to the other starches, while the high amylose rice starch had the highest S' value. Similar phenomena can be observed for the cooked rice grains in column A. For other starches, minor differences exist between the relative positions of the S' curves of purified starch and cooked rice grains.

Table 1 shows the values of the Avrami parameter n and k for starch retrogradation in purified rice starches and cooked rice grains. For starches in cooked rice grains, the n_1 value ranges from 0.551 (high amylose) to 1.565 (93-124), and the k_1 value ranges from 0.043 (93-124) to 0.970 (high amylose). For purified starches, the n_2 value ranges from 0.565 (high amylose) to 1.474 (93-124), and the k_2 value ranges from 0.027 (93-124) to 0.868 (high amylose). Our result is consistent with the work of Fan and Marks (29), who used DSC analysis and reported n values of 0.63–1.53 and k values of 0.181–0.917 for cooked rice starch and rice flours with amylose contents ranging from 15.1% to 28.4%.

S'_0 and Amylose Content. Figure 3 shows the relationship between initial S' (S'_0) and amylose content. It is shown that S'_0 values of purified rice starch and cooked rice grains are

Table 2. Pearson Correlation among Parameters Describing the Structure and Retrogradation Kinetics of Rice Starches^a

| | n_1 | k_1 | n_2 | k_2 | DP ₁ (DP) | DP ₂ (DP) | P ₁ /P ₂ | AM ₀ (%) | ΔAM (%) |
|--------------------------------|-------|----------|----------------------------|----------------------------|-------------------------|-------------------------|--------------------------------|------------------------|------------|
| n_1 | 1.000 | -0.854** | 0.823**^b | -0.718** | 0.566* | -0.473* | 0.643** | -0.874** | -0.459 |
| k_1 | | 1.000 | -0.780** | 0.956**^b | -0.274 | 0.721** | -0.352 | 0.656** | 0.189 |
| n_2 | | | 1.000 | -0.774** | 0.305 | -0.482* | 0.500* | -0.836** | -0.344 |
| k_2 | | | | 1.000 | -0.164 | 0.777** | -0.237 | 0.539* | 0.0499 |
| DP ₁ (DP) | | | | | 1.000 | -0.107 | 0.832** | -0.622** | -0.412 |
| DP ₂ (DP) | | | | | | 1.000 | -0.102 | 0.311 | 0.152 |
| P ₁ /P ₂ | | | | | | | 1.000 | -0.827** | -0.565* |
| AM ₀ (%) | | | | | | | | 1.000 | 0.652** |
| ΔAM (%) | | | | | | | | | 1.000 |

^aSignificant at $P < 0.05$. ^{**}Significant at $P < 0.01$. ^aSymbols have the same meaning as in Table 1. ^bCoefficients indicating the relationship between starch retrogradation behaviors in cooked rice grains and in purified rice starch.

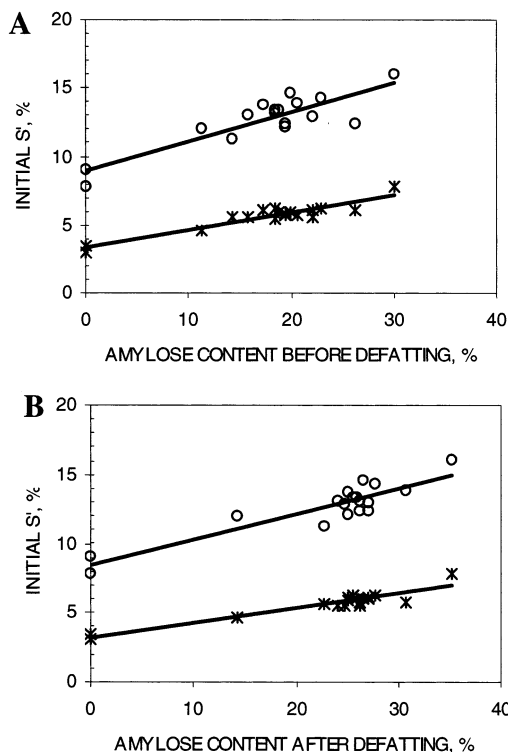


Figure 3. Relationships between the initial relative solid content S' (S'_0 , %) and amylose content before (A) and after (B) defatting of starch as investigated in cooked rice grains (○) and in purified rice starch (*).

positively related to amylose content both before and after defatting. It was indicated that the S'_0 and S'_t values of starch sample are comprised of the contributions from both amylose and amylopectin. The contribution from amylose remains constant during the whole retrogradation process, whereas the contribution from amylopectin increases during storage (37).

DISCUSSION

Similarity of Retrogradation Behavior between Purified Starches and Starches in Cooked Rice Grains. Pearson correlation was applied to parameters describing starch structure and retrogradation kinetics. The original coefficients are shown in Table 2. Because of the effects of other variables, caution should be taken in explaining the coefficient between a certain pair of variables. We would not go further than indicating that the amylopectin crystallization rate constants k_1 and k_2 are strongly correlated with each other (coefficient 0.956). The same is true with n_1 and n_2 (coefficient 0.823). Thus, a strong similarity exists between the retrogradation behavior of rice starches in cooked rice grains and those in the purified form.

Table 3. Partial Correlation among Parameters Describing Amylopectin Chain Length Distribution and Retrogradation Kinetics of Rice Starches^a

| | DP ₁ (DP) | DP ₂ (DP) | P ₁ /P ₂ |
|-------|----------------------|----------------------------|--------------------------------|
| n_1 | 0.064 | -0.435 | -0.286 |
| k_1 | 0.246 | 0.763**^b | 0.468^b |
| n_2 | -0.567* | -0.450 | -0.677** |
| k_2 | 0.289 | 0.828**^b | 0.468^b |

^aSignificant at $P < 0.05$. ^{**}Significant at $P < 0.01$. ^aSymbols have the same meaning as in Table 1. ^bCoefficients discussed in the text.

Partial Correlation Used in Describing the Structure–Retrogradation Relationship. In starch systems, due to the effects of other variables, the actual relationship between any two variables may not be reflected by the original coefficient obtained from Pearson correlation. In the current study, the relationships among four retrogradation parameters, n_1 , n_2 , k_1 , and k_2 , and five structure parameters, DP₁, DP₂, P₁/P₂, AM₀, and ΔAM, were investigated by partial correlations. Generally, partial correlation measures the strength of the relationship between two variables while controlling for the effect of one or more other variables.

Effect of Amylopectin Structure on Retrogradation Behavior. The relationship between retrogradation behavior and amylopectin structure was investigated while controlling for the effects of amylose and lipid. Partial correlation was performed among retrogradation kinetics parameters (n_1 , n_2 , k_1 , and k_2) and parameters describing the amylopectin chain length distribution (DP₁, DP₂, and P₁/P₂) while amylose content after defatting (AM₀) and the content of amylose associated with lipid (ΔAM) were controlled. The results in Table 3 indicate that, when the effects from amylose and lipid are controlled, the crystallization rate constants of amylopectin in both cooked rice grains and purified rice starch, k_1 and k_2 , have a significant positive relationship with the peak value of short chain length, DP₂ (coefficients of 0.763 and 0.828, respectively). Gidley and Bulpin (38) indicated that a minimum chain length of DP₁₀ is required for the formation of double helices. Since short chains in peak 2 constitute the major portion of external chains forming double helices, we consider that a higher value of DP₂ facilitates the formation of double helices and thus results in a higher retrogradation rate. Positive coefficients also exist between P₁/P₂ and k_1 (0.468) and k_2 (0.468) when the effects of AM₀ and ΔAM are controlled.

Effect of Amylose Content on Retrogradation Behavior. The coefficients between amylose content after defatting (AM₀) and amylopectin crystallization rate constant in cooked rice grains and rice starch (k_1 and k_2) are both positive (0.760, $P < 0.01$, and 0.748, $P < 0.01$) when the effects of amylopectin

structure (DP₁, DP₂, P₁/P₂) and lipid (Δ AM) are controlled. Thus, higher amylose content corresponds to higher retrogradation rate of amylopectin. This result is consistent with the work of Klucinec and Thompson (39). They reported that gels of amylose with wx starch (amylopectin) developed a higher retrogradation enthalpy than corresponding amylopectin without amylose when the retrogradation enthalpy values were normalized to the amylopectin content. They suggested that the amylose might interact with external chains of amylopectin. Our hypothesis is that the amylose in starch may be related to the crystallite formation of amylopectin during retrogradation, by affecting either the number of nuclei or the orientation of double helices formed by amylopectin external chains.

Meanwhile, AM₀ has a strong negative relationship with n_1 and n_2 (coefficients -0.808 , $P < 0.01$, and -0.894 , $P < 0.01$), which indicates the close relationship between amylose content and the crystallite growth mode of amylopectin.

Effect of Lipid on Retrogradation Behavior. The coefficients between the content of amylose associated with lipid (Δ AM) and the amylopectin crystallization rate constant in rice starch and cooked rice grains (k_1 and k_2) are all negative (-0.553 , $P < 0.05$, and -0.678 , $P < 0.01$) when the effects of amylopectin structure (DP₁, DP₂, P₁/P₂) and amylose content after defatting (AM₀) are controlled. The lipid content was reported to be 1.27% for normal rice and 0.65% for purified normal rice starch (17). We consider that, by associating with a portion of amylose, the lipid decreases the amount of amylose available to interact with the external chains of amylopectin, thus decreasing the retrogradation rate of amylopectin.

ABBREVIATIONS AND NOMENCLATURE

PNMR, pulsed nuclear magnetic resonance; DP, degree of polymerization; AM, amylose content before defatting; AM₀, amylose content after defatting; Δ AM, content of amylose associated with lipid; HPSEC, high-performance size-exclusion chromatography; DP₁, chain length (in DP) at the climax of the left peak (peak 1) of the HPSEC chromatogram; DP₂, chain length (in DP) at the climax of the right peak (peak 2) of the HPSEC chromatogram; P₁/P₂, ratio between the amount of long chains and short chains; S' , relative solid content measured by PNMR; S'_0 , initial S' of retrogradation monitoring; S'_{Retro} , S' at the end of the storage period; S'_t , S' at storage time t ; n , Avrami parameter related to the crystallite growth mode; k , Avrami parameter describing the crystallization rate constant.

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