Pulsed Nuclear Magnetic Resonance (PNMR) Study of Rice Starch Retrogradation

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ABSTRACT

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Although pulsed NMR (PNMR) has been used for qualitative study of starch retrogradation in selected systems, validation is necessary for its application to new systems. PNMR was used to analyze the retrogradation of rice starches in purified form, in rice flour, and in cooked rice grains. The standard curves between the relative solid content (S´, %) by PNMR and the percentage of gelatinized starch (GS, %) were determined for common rice flour, common rice starch, and waxy rice starch at different moisture contents. The coefficients of linear regression for these curves (R^2) were all >0.997. Starches with different amylose contents were tested for S´ values at the stages of freshly gelatinized, retrograded (4°C, 18 days), and reheated (90°C, 20 min). The S´ of reheated starch ($S´_{reheat}$) was similar to the S´ of freshly gelatinized starch ($S´_0$), so we concluded that the increase in S´ during storage corresponded to amylopectin retrogradation. The effect of moisture content on retrogradation of rice starch, rice flour, and cooked rice grains was studied by PNMR, and the data were interpreted using the Avami equation. Decreasing the moisture content increased the rate of retrogradation and led to a higher parameter k and a lower parameter n. For moisture content in the range studied, PNMR can be used to follow amylopectin retrogradation of different rice starch systems.

Rice products are staple foods, especially in Oriental countries. Both nutritional and sensory properties have been used to determine the quality of rice products. The retrogradation of rice starch during storage is the major reason for the aging of rice products and deterioration of desirable qualities. To understand the aging process for these products, appropriate methods are needed to monitor the retrogradation of starch in food systems. Starch retrogradation has been studied extensively by several methods. DSC monitors the enthalpy increase corresponding to the ordering process of amylopectin external chains (Jane et al 1999; Lai et al 2000; Tako and Hizukuri 2000; Tsai and Lii 2000). Dynamic viscoelasticity analysis measures the increase in storage modulus (G') that describes the development of a gel network (Otobe et al 1995; Morikawa and Nishinari 2000; Tako and Hizukuri 2000). Enzymatic analysis (Tsuge et al 1992; Kim et al 1997) is based on the susceptibility of starch to attack by certain kinds of amylase, which could be related to the extent of ordered structures formed during the storage process. X-ray analysis (Jagannath et al 1998; Jouppila et al 1998) gives direct measurements of the degree of crystallinity of polysaccharide chains. When comparing the data from differential scanning calorimetry (DSC) and X-ray results, one should bear in mind that crystallites in the starch system detectable using DSC would not necessarily show up as crystalline regions by X-ray diffraction if the size remained below a certain threshold. Infrared spectroscopy was used to investigate the changes in starch structure on a short-range molecular level in which selected peaks were used to characterize the more organized part of starch, the amorphous part of starch, and the water (Wilson et al 1991; Ogawa et al 1998; Smits et al 1998). Literature of instrumental studies on the storage of rice products are fewer and tend to focus on mechanical and thermal analysis (Lima and Singh 1993; Villareal et al 1997; Perdon et al 1999; Riva et al 2000). Perdon et al (1999) indicated that starch retrogradation measured by DSC showed positive linear trends with firmness for rice cultivars studied at all storage temperatures and with stickiness for cultivar Bengal stored at –13°C and 3°C and for cultivar Cypress stored at 3°C and 20°C.

Pulsed nuclear magnetic resonance (PNMR) has been used for studies on the retrogradation of starch and cereal foods (Teo and Seow 1992; Wursch and Gumy 1994; Lebotlan and Desbois 1995; Seow and Teo 1996; Farhat et al 2000; Teo et al 2000). In contrast to many other methods, PNMR is rapid, reproducible, and nondestructive, which is important in monitoring the retrogradation process of common rice products including rice pasta, noodles, and cooked grains.

In PNMR analysis, the relative solid content value (S´) in a starch system has been commonly used to describe the degree of retrogradation. The bi-gaussian fitting performed on the free induction decay (FID) curve of spin-spin relaxation time T_2 has been used to calculate the true S´ signal coming from the solid phase that could not be directly obtained because of the dead time of the probe (Lebotlan and Desbois 1995). The relative solid content given by the instrument was also directly used to characterize the retrogradation behavior (Teo and Seow 1992).

The Avrami equation has been used to describe polymer crystallization kinetics during an isothermal process (Wunderlich 1976). This equation has been applied in research of starch retrogradation for characterizing the crystallite formation in gelatinized starch systems (McIver et al 1968; Colell et al 1969; Longton and LeGrys 1981; Wong and Lelievre 1982; Fearn and Russell 1982, 1983a–c; Bulkin et al 1987; Marsh and Blanshard 1988; Inouchi et al 1991; Zhang and Jackson 1992; Mita 1992; Wu and Eads 1993; Baik et al 1997; Armero and Collar 1998; Jouppila et al 1998; Lim et al 1998; Liu and Thompson 1998; Riva et al 2000; Indrani et al 2000; Lai et al 2000). Although it was often used in a descriptive way for comparison among starches or treatments, the requirement for the linear relationship between the physical properties of data used in the model and the amount of crystallites was often neglected.

The establishment of a suitable analytical system is essential for realizing the application of PNMR to monitor linearly the retrogradation of rice products and for subsequent potential for kinetic analysis. In this study, the changes in relative solid content S´ of the system were followed by PNMR, and the data were used to analyze rice starch retrogradation. The research addresses three questions: 1) Does the relative solid content S´ by PNMR have a linear relationship with the crystallite content in the starch system, so the S´ value can be used in Avrami kinetic analysis? 2) Which portion of crystallite formation in a retrograded starch system is responsible for the S´ changes during the storage? 3) Does the data for starch retrogradation obtained using PNMR have a good fit to the Avrami equation?

MATERIALS AND METHODS

Milled rice, rice flour, rice starch, and rice amylopectin were used. The milled rice from different cultivars were kindly provided by the Chinese Institute of Agriculture (Beijing, China).

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Rice flour suspensions were prepared from milled rice soaked in deionized water (×5, w/w) using an electric homogenizer until the suspension passed through a 100-mesh sieve. The precipitate after centrifugation was then dried at 40°C until moisture content was <8%. For rice starch, rice flour was soaked in 0.4% NaOH solution (×5, w/w) for 48 hr and washed by deionized water repeatedly until pH 7 was reached. The starch precipitate was dried at 40°C until moisture content was <6%.

Amylopectin from rice starch was prepared according to the method of Takeda et al (1986, 1987). For the measurement of moisture content, a sample was first dried at 40°C in an oven for 48 hr to remove most of the water, and then was heated at 105°C to constant weight when the samples were weighed at an interval of 12 hr.

Amylose Content

The defatting of starch was performed according to Takeda et al (1986). Amylose content was measured as 100 mg 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 1.0 mL of iodine reagent (0.30% I_2 + 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.

Avrami Equation

In the Avrami equation, $V = 1 - \mathrm{EXP}\,(-kt^n)$, V is the crystallite percentage of the limiting value at storage time t. The term k is the crystallization rate constant and, in theory, it is related to the number of nuclei and the crystallite growth rate. The term n is related to the crystallite growth mode. Both n and k can be obtained by linear regression of equation $\ln(-\ln(1 - V)) = \ln k + n \ln t$ derived from the original Avrami equation. When the requirement for the linear relationship between PNMR signal S´ and the crystallite amount is satisfied, V can be calculated as:

$$(S'_t - S'_0)/(S'_{retro} - S'_0)$$

where S'_0 is the initial S´ for retrogradation monitoring, S'_{retro} is the S´ at the end of storage period, and S'_t is the S´ at storage time t.

PNMR

Software for an experimental definition module (EDM 110A) was used to measure the relative solid content of starch systems by PNMR (Minispec PC120, Bruker Corp.: Billerica, MA). The size of the test tube was 180 mm × 10 mm. Magnetic field was 20 MHz. Detector temperature was 40°C. Nine scans were performed for each test. The digital offset φ value was 0.135. The attenuation value was 38. The calibration factor f was 1.475, determined by mineral oil standards with predetermined relative solid content values.

A 9-scan PNMR test for a single sample was completed in 15 sec. On placing a sample previously at 4°C into the 40°C PNMR detector, the S´ data obtained was essentially the same for two consecutive 9-scan tests. Based on this evidence, we consider that the temperature change of the sample was not critical to the measurement of S´ value in the period up to 30 sec after the PNMR test tube was taken from the 4°C water bath and put in the 40°C detector.

S´ and GS Changes During Treatment

Common waxy rice starch, 93-124 waxy rice starch, Jiran rice starch, Huwan rice starch, Dalian rice starch, high-amylose rice starch, and amylopectin isolated from Huwan rice starch were dispersed in deionized water at 100°C for 20 min to form 30% (w/w) starch gels which were then stored at 4°C. S'_0 was measured after the freshly gelatinized starches were stabilized at 4°C for 30 min. S'_{retro} was measured at the end of 18 days of storage at 4°C. After storage, the retrograded samples were reheated at 90°C for 20 min. The S'_{reheat} was then measured after 30 min of equilibration at 4°C. All samples were tested in duplicate and the mean values were used in analysis.

Huwan rice flour suspensions with moisture contents of 61.7, 65.0, 67.3, 70.0, and 72.7%; Huwan rice starch suspensions with moisture contents of 67.7, 72.3, and 76.9; and 93–124 waxy rice starch suspensions with moisture contents of 66.0, 69.1, and 72.9% were prepared. Half of each suspension was cooked at 100°C for 20 min. After cooling, deionized water was added to the gelatinized system to compensate for moisture lost. The percentage of gelatinized starch GS is defined as the percentage of gelatinized starch in the total amount of starch, both for rice flour and rice starch systems. To prepare mixtures with GS of 20, 40, 60, and 80%, the gelatinized gel was mixed with the corresponding ungelatinized suspension at ratios of 1:4, 2:3, 3:2, and 4:1 using a mortar and pestle. Moisture compensation was also needed during mixing. The mixture was then injected into the bottom of a PNMR test tube to reach a sample height of 60 mm. Care was taken to not introduce air bubbles in the starch gel. The systems including mixtures, gelatinized gels (GS 100%), and ungelatinized suspensions (GS 0%) were all placed in a 4°C water bath for 30 min for equilibration. After equilibration, the S´ of each sample was measured using PNMR. All samples were tested in triplicate and the mean values were used in analysis. For each system, the standard curves for S´ and GS under various moisture contents were prepared.

Retrogradation of Starch Systems

Huwan rice starch suspensions with moisture contents of 60.0, 64.0, 67.2, 70.0, and 72.3%; and Huwan rice flour suspensions with moisture contents of 60.0, 63.0, 66.0, 69.2, and 72.4%, were prepared in beakers and transferred into PNMR tubes. After sealing and shaking, the test tubes were heated in a boiling water bath for 20 min. After gelatinization, the samples were stabilized in a 4°C water bath for 30 min and the S'_0 was measured using PNMR. For rice starch samples, the S´ values during the 4°C storage were also measured after 1, 2, 4, 5, 6, 8, 9, 10, 12, 13, and 15 days. For rice

TABLE I
Relative Solid Content (S´, %) Values[a] for 30% (w/w) Starch Samples at Different Stages in a Gelatinization-Retrogradation-Reheating Treatment[b]

	Amylose Content (%)						
Sample	Before Defatting	After Defatting	S'_0	S'_{retro}	S'_{reheat}	ΔS´	ΔS´´
Huwan amylopectin	0.0 ± 0.6	0.0 ± 0.5	3.19 ± 0.03	8.13 ± 0.06	3.16 ± 0.02	4.94	4.94
Common waxy rice starch	0.0 ± 1.2	0.0 ± 1.0	3.11 ± 0.03	11.72 ± 0.07	3.13 ± 0.02	8.61	8.61
93-124 waxy rice starch	0.0 ± 1.1	0.0 ± 0.9	3.42 ± 0.05	9.67 ± 0.07	3.48 ± 0.04	6.25	6.25
Jiran rice starch	11.3 ± 0.8	14.3 ± 0.9	4.58 ± 0.08	10.39 ± 0.11	4.63 ± 0.06	5.81	6.78
Huwan rice starch	19.5 ± 1.3	26.2 ± 1.3	5.92 ± 0.07	12.12 ± 0.09	5.98 ± 0.07	6.20	8.40
Dalian rice starch	22.1 ± 1.6	24.8 ± 1.4	5.64 ± 0.06	12.15 ± 0.10	5.68 ± 0.05	6.51	8.66
High amylose rice starch	30.0 ± 1.9	35.2 ± 1.7	8.04 ± 0.11	16.88 ± 0.13	8.10 ± 0.08	8.84	13.64

[a] S'_0 measured after freshly gelatinized or dispersed starches were stabilized at 4°C for 30 min; S'_{retro} measured after 18 days of storage at 4°C; S'_{reheat} measured when retrograded starches stored at 4°C for 18 days were reheated at 90°C for 20 min, and then stabilized at 4°C for 30 min. $\Delta S' = S'_{retro} - S'_0$, describing S´ changes during retrogradation. $\Delta S'' = \Delta S'/(1 - \text{amylose content after defatting})$, describing S´ changes per unit amount of amylopectin.

[b] Samples measured in duplicate and reported as mean value ± standard deviation.

flour samples, the S´ were also measured every 24 hr during the 13 days of storage at 4°C. For the preparation of cooked rice grains in the PNMR tubes, the milled rice grains mixed with different amounts of water were steamed at normal pressure for 20 min and cooled to room temperature. S´ was measured after 30 min of equilibration at 4°C and every 24 hr during the nine days of storage at 4°C. All samples were tested in duplicate and the mean values were used for analysis.

RESULTS

S´ Changes During the Gelatinization-Retrogradation-Reheating Treatment

Table I shows the S´ changes during the process of gelatinization-retrogradation-reheating for starches with different amylose contents. Generally, the S'_{reheat} values were essentially the same as S'_0 for all the samples, which indicates that the increase in S´ during retrogradation could be eliminated by heating at 90°C for 30 min. Higher amylose content resulted in higher S'_0 and S'_{reheat}. For example, the S'_0 of 93-124 waxy rice starch, Jiran starch, and high-amylose starch (with amylose contents of 0.0, 11.3, and 30.0% before defatting) were 3.19, 4.58, and 8.04%, respectively. Similar phenomena were observed for other starches. Both ΔS´ (the increase of S´ value during storage) and ΔS´´ (the increase of S´ value per unit amount of amylopectin) tended to increase with the increase of amylose content. For example, ΔS´ was 4.94% for purified amylopectin and 8.84% for high-amylose rice starch. Meanwhile, ΔS´´ was 4.94% for amylopectin and 13.64% for high-amylose rice starch. Common waxy rice starch is a special case because ΔS´ and ΔS´´ values were higher than most other starches.

Standard Curves for S´ and GS

Figures 1–3 give the standard curves between relative solid content (S´) and the percentage of gelatinized starch (GS) for Huwan rice flour, Huwan rice starch, and 93-124 waxy rice starch. The moisture content affected the equation of the standard curves. For Huwan rice flour, when the moisture contents were 61.7, 65.0, 67.3, 70.0, and 72.7%, for standard curves R^2 = 0.9978, 0.9984, 0.9995, 0.9986, and 0.9991, respectively. For Huwan rice starches, when the moisture contents were 67.7, 72.3, and 76.9%, R^2 = 0.9985, 0.9999, and 0.9996, respectively; For 93–124 wax rice starch, when the moisture contents were 66.0, 69.1, and 72.9%, R^2 = 0.9984, 0.9995, and 0.9995, respectively. For a particular starch, an increase of moisture content shifted the curve downward and produced a decreased slope.

Effect of Moisture Content on Starch Retrogradation

Figures 4–6 describe the retrogradation behaviors for Huwan rice starch, rice flour, and cooked rice grains with different moisture contents. Table II shows the application of Avrami equation to these systems. Generally, $R^2 \approx 1$ (0.972–0.994), which indicates that Avrami equation can give a satisfactory description on the growth of crystallites, as monitored by PNMR. Two conditions applied to all starch systems studied. 1) The Avrami exponent n increased when the moisture content increased. For rice starch, n value increased from 0.90 to 1.35 when moisture content increased from 60.0 to 72.3%; for rice flour, n value increased from 1.00 to 1.32 when moisture content increased from 60.0 to 72.4%. For cooked rice grains, n value increased from 1.05 to 1.14 when moisture content increased from 63.2 to 66.1%. All n values were <2. 2) The crystallite growth rate constant k decreased with increased moisture content. For rice starch, the k value decreased from 0.360 to 0.063 when moisture

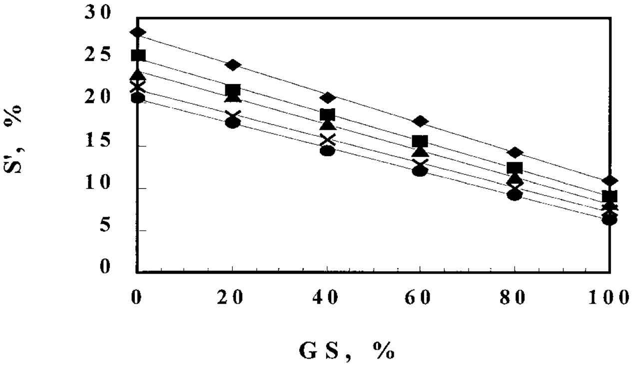

Fig. 1. Standard curves for relative solid content (S´) and % of gelatinized starch (GS) for Huwan rice flour with moisture contents of 61.7% (◆), R^2 = 0.9978; 65.0% (■), R^2 = 0.9984; 67.3% (▲), R^2 = 0.9995; 70.0% (×), R^2 = 0.9986; and 72.7% (●), R^2 = 0.9991.

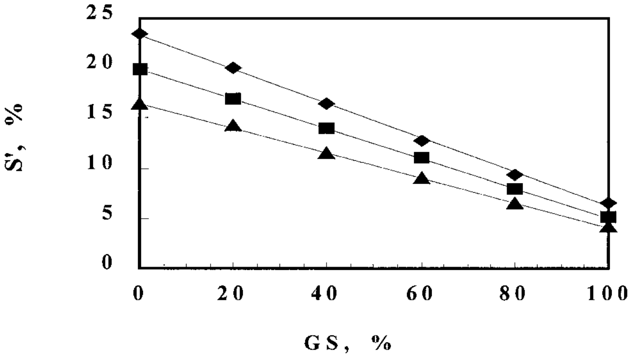

Fig. 2. Standard curves for relative solid content (S´) and % of gelatinized starch (GS) for Huwan rice starch with moisture contents of 67.7% (◆), R^2 = 0.9985; 72.3% (■), R^2 = 0.9999; and 76.9% (▲), R^2 = 0.9996.

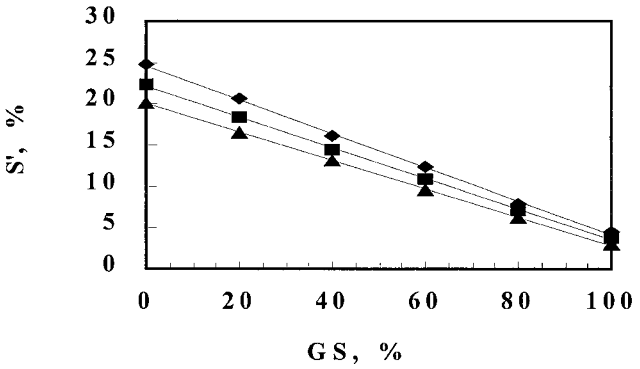

Fig. 3. Standard curves for relative solid content (S´) and % of gelatinized starch (GS) for 93-124 waxy rice starch with moisture contents of 66.0% (◆), R^2 = 0.9984; 69.1% (■), R^2 = 0.9995; and 72.9% (▲), R^2 = 0.9995.

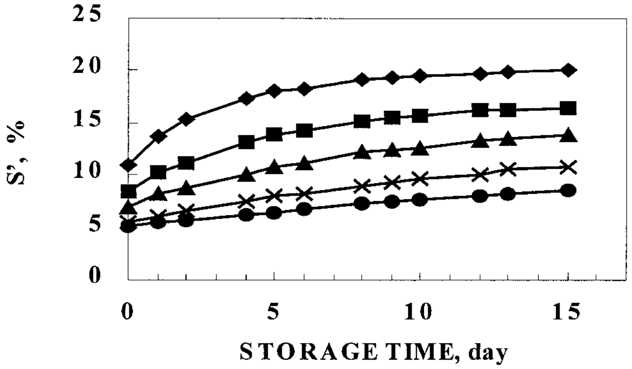

Fig. 4. Effect of moisture content on retrogradation of Huwan rice starch during storage at 4°C for 15 days. Moisture contents were 60.0% (◆), 64.0% (■), 67.2% (▲), 70.0% (×), and 72.3% (●), respectively.

content increased from 60.0 to 72.3%. For rice flour, the *k* value decreased from 0.375 to 0.102 when moisture content increased from 60.0 to 72.4%. For cooked rice grains, the *k* value decreased from 0.372 to 0.293 when moisture content increased from 63.2 to 66.1%.

DISCUSSION

S′ of PNMR of Retrogradation

In PNMR, S′ of a certain sample is a statistical description of the microenvironment of all protons. The spin-spin relaxation time (T_2) of individual proton after the 90o pulse of rotation magnetization is determined by the microenvironment. In a starch-water system, the protons are part of water molecules and starch chains, and both may exist in different regions or phases. Even though the system cannot be as simple as a physical mixture of liquid and solid, the simplified description of the system by S′ can be used to follow the retrogradation process quantitatively, provided that there is a linear relationship between S′ and the crystallite content in the system.

As shown in Fig. 7, measuring the solid content of a simplified solid-liquid mixture is based on the idea that the free induction decay (FID) curve following a 90o pulse can be considered to be two superimposed components, one due to the solid part of the sample and the other to the liquid part. The decay of each component is governed by the respective spin-spin relaxation time (T_2). For the solid, T_2 is of the order of tens of microseconds while for the liquid it is much longer, in the millisecond range (Anonymous 1989).

The signal amplitude at t_0 is proportional to the number of protons in the sample (Anonymous 1989). Therefore, SA_0 is proportional to the total amount of sample, SA_2 is proportional to the amount of liquid, and the difference ($SA_0 - SA_2$) is proportional to the amount of solid. Due to the dead time of the receiver (9 μsec at 20 MHz), it is impossible to measure SA_0 at t_0. For liquid, this is not a problem because the amplitude at t_2 is virtually the same as the initial amplitude SA_2. For solids, however, it is necessary to multiply the amplitude $SA_1 - SA_2$ by a previously determined factor (*f* factor) to obtain $SA_0 - SA_2$. Amplitude measurements are made at t_1 (11 μsec) and t_2 (70 μsec), which have been factory programmed. Thus, the relative solid content S′ measured by PNMR is:

$$S' = (SA_1 - SA_2)f \,/\, [(SA_1 - SA_2)f + SA_2 + \varphi] \times 100$$

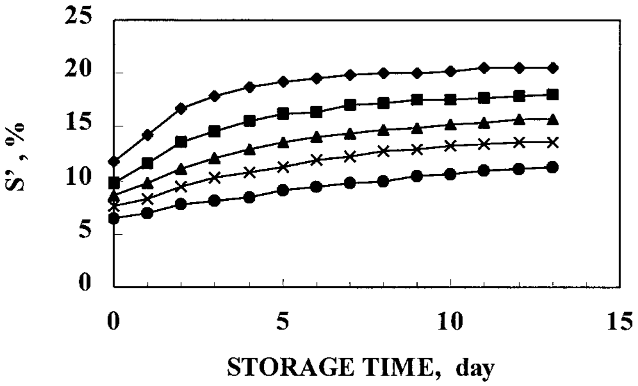

Fig. 5. Effect of moisture content on retrogradation of Huwan rice flour during storage at 4°C for 13 days. Moisture contents were 60.0% (◆), 63.0% (■), 66.0% (▲), 69.2% (×), and 72.4% (●), respectively.

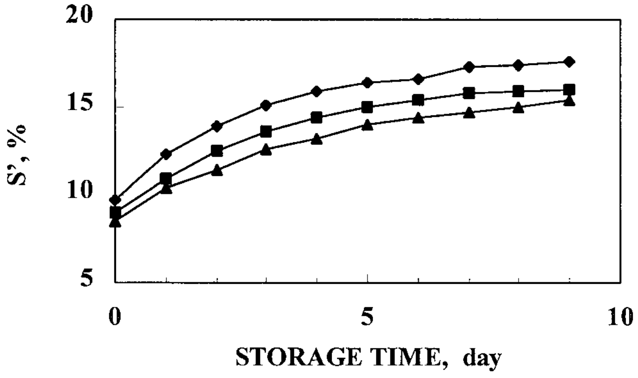

Fig. 6. Effect of moisture content on retrogradation of cooked Huwan rice grains during storage at 4°C for 9 days. Moisture contents were 63.2% (◆), 65.0% (■), and 66.1% (▲), respectively.

In the equation, φ is the digital offset corresponding to the individual diode detector. The *f* factor is used to calibrate the system so that the S′ for standard samples are equal to predetermined values. In the determination of the calibration factor *f*, because no starch standard with predetermined relative solid content was available, the mineral oil standards provided by the PNMR equipment manufacturer (Bruker Corp.) were used. The *f* factor thus determined was 1.475. The use of oil standards in instrument calibration and the simplified description of the starch-water system using S′ necessitate the detailed investigation between the relative solid content S′ and the percentage of gelatinized starch GS.

From the linear relationship between S′ and GS, we concluded that S′ is related linearly to crystallite content in all starch mixture systems studied. Note that neither nonstarch impurities in Huwan rice flour, nor amylose in Huwan rice starch affected the linear

TABLE II
Avrami Equation for Retrogradation of Huwan Rice Starch Systems[a]

	Moisture Content %	R^2 [b]	*n* [c]	*k* [d]
Rice starch	60.0 ± 0.1	0.994	0.90	0.360
	64.0 ± 0.1	0.986	1.04	0.219
	67.2 ± 0.1	0.974	1.08	0.160
	70.0 ± 0.2	0.976	1.15	0.107
	72.3 ± 0.1	0.987	1.35	0.063
Rice flour	60.0 ± 0.1	0.981	1.00	0.375
	63.0 ± 0.1	0.977	1.11	0.252
	66.0 ± 0.1	0.972	1.24	0.165
	69.2 ± 0.2	0.991	1.30	0.129
	72.4 ± 0.2	0.978	1.32	0.102
Cooked rice grains	63.2 ± 0.2	0.981	1.05	0.372
	64.2 ± 0.2	0.994	1.14	0.302
	66.1 ± 0.1	0.987	1.14	0.293

[a] Samples measured in duplicate and reported as mean ± standard deviation.
[b] Linear regression coefficient.
[c] Avrami exponent of time.
[d] Avrami crystallite growth rate constant.

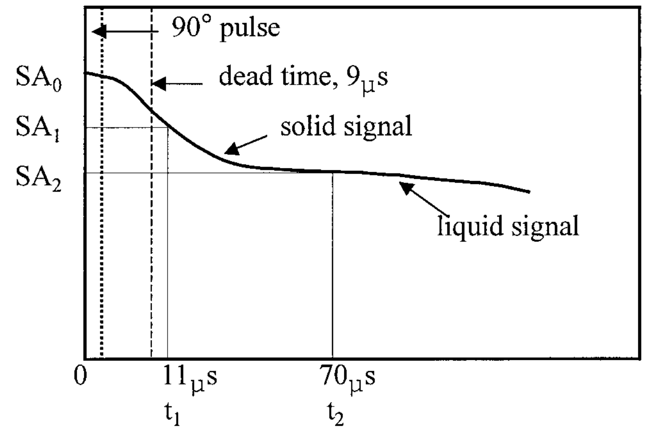

Fig. 7. Free induction decay (FID) curve after a 90° pulse. SA_0, SA_1, and SA_2 are signal amplitudes at times $t_0 = 0$ μsec, $t_1 = 11$ μsec, and $t_2 = 70$ μsec after the pulse (Anonymous 1989). The *x*-axis is time after the pulse, and the *y*-axis is the signal amplitude received by the detector.

relationship between S´ and GS. When the PNMR was applied in an actual starch retrogradation system in which the distribution of crystallites and related protons were different from the starch mixtures used in this study, the number of protons, rather than their distribution in the system, determined the S´ value of the PNMR. The satisfactory fit of S´ in Avrami equation for actual retrograded starch systems gives further support for the use of S´ in monitoring the retrogradation process of rice starch. Thus, we concluded that S´ can be used to follow the degree of retrogradation in starch systems.

ΔS´ and Amylopectin Retrogradation

To explain how $S´_0$ and $S´_{reheat}$ are essentially the same for all starches, we assumed that crystallites formed by amylopectin in starch during the storage period could be melted after the gelatinization-retrogradation-reheating treatment. Thus, the change of relative solid content S´ ($\Delta S´ = S´_{retro} - S´_0$) was basically caused by the ordering of amylopectin chains. The fact that $S´_0 = S´_{reheat}$ suggests that at the time of $S´_0$ measurement (following 30 min of equilibration at 4°C), the aggregation of amylose had been mostly completed, whereas the extent of crystallite formation by amylopectin was still negligible. Gidley (1989) indicated that the aggregation of amylose and long-chain glucans results in gel formation and can be accelerated by increasing the concentration and decreasing the temperature. Biliaderis (1990) showed that the storage modulus of amylopectin gel (40%, w/w) reaches the stabilized value much more slowly than that of amylose gel with much lower concentration (5%, w/w).

It is useful to compare S´ values for Huwan rice starch and amylopectin isolated from this starch. When amylose was removed from the starch (as for purified amylopectin), $S´_0$ changed from 5.92% for starch to 3.19% for amylopectin, and $S´_{retro}$ changed from 12.12% for starch to 8.13% for amylopectin. Thus, ΔS´ ($S´_{retro} - S´_0$) changed from 6.20 to 4.94%, and ΔS´´ (ΔS´ value normalized by amylopectin amount) changed from 8.40 to 4.94% upon removal of amylose. Note that the removal of amylose correlated with less retrogradation of amylopectin. A similar phenomenon can also be observed when the amylose contents (both before and after defatting) and ΔS´ (or ΔS´´) are compared among Jiran, Huwan, Dalian, and high-amylose rice starches. Klucinec and Thompson (2002) 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 helixes formed by amylopectin external chains. Among various starch systems, common waxy rice starch is a special case where ΔS´ and ΔS´´ are higher than most other starches. Amylopectin fine structure may be responsible for S´ differences among Huwan amylopectin, common waxy, and 93-124 waxy rice starches. More starch fine structure and crystallization kinetic studies are needed to elucidate the effect of amylose content and amylopectin structure on the retrogradation of amylopectin.

In concentrated starch gel systems with sufficient water, the retrogradation process can be largely influenced by the moisture content. The fact that for all starch systems studied, the Avrami exponent $n < 2$ suggests that the recrystallization process of amylopectin may be due to a three-dimensional fibril crystallization growth pattern with both athermal and thermal nucleation process (Wunderlich 1976). It is possible that the moisture content may affect the relative location of amylose and amylopectin chains and amylopectin branching points, thus influencing the nucleation pattern for amylopectin crystallization, and determine the ratio between the athermal and thermal mechanisms responsible for the *n* value. The Avrami *k* value decreased as a function of moisture content for all the three systems. The dilution effect of water may decrease the concentration of nuclei in the system and extend the migrating distance among the chains needed in forming double helixes starting from the branching points, thus resulting in a decreased rate constant *k*.

CONCLUSIONS

For kinetic analysis using the Avrami equation, the measured signal must be linearly related to the growth of crystallites. For this purpose, the signal S´ of PNMR was tested for its linear relationship with the percentage of gelatinized starch in model mixture systems. We assumed that the degree of retrogradation in actual rice starch systems can also be linearly monitored by S´. The crystallite growth of amylopectin accounts for the increase of S´ under the experimental conditions used. The growth of amylopectin crystallites may be related to a three-dimensional fibril crystallization growth pattern. The Avrami exponent *n* increased and crystallization rate constant *k* decreased when the moisture content increased, which may be explained by the effects of water on the nucleation mechanism, the concentration of nuclei, and the migration distance among amylopectin external chains. More information on how the amylose content affects amylopectin crystallite growth is needed to elucidate the role of amylose in the retrogradation of amylopectin in starch systems.

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