

## Effects of Urea Concentration on Thermal and Rheological Properties of Rice Starches

Meng-I Kuo<sup>1,2</sup> and Ya-Jane Wang<sup>1,3</sup>

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Starch is an important ingredient of many processed foods. When starch is heated in excess water, it loses its ordered molecular arrangement and swells irreversibly; this is termed gelatinization. These changes are responsible for the unique characteristics of starch-containing products. Because the purpose of incorporating starch in various applications is often related to the viscosity development upon gelatinization, a thorough understanding of the basis for the increase and maintenance of viscosity is critical.

The gelatinization properties and rheological behavior of starch during heating are results of several cooperative factors: the structure of the hydrated starch molecules (Asaoka et al 1984, 1985; Tester and Morrison 1990a,b; Shi and Seib 1992; Sasaki and Matsuki 1998; Jane et al 1999), interactions between lipids and amylose (Morrison 1995), and noncovalent bondings between starch molecules (Tako and Hizukuri 1997, 1999). Thus, any changes in these structural characteristics would result in subsequent changes in the rheological behavior of starch (Mita and Matsumoto 1981).

Hydrogen bonding has been postulated to play a critical role in starch gelatinization. Hydrogen bonds are continuously broken and reformed throughout the course of heating (Caesar 1950). Water and starch molecules form a network through hydrogen bonding, which is responsible for viscosity increase. Dimethyl sulfoxide (DMSO) or DMSO-based solvents are widely used to solubilize starch for structural characterization (Solorza-Feria et al 2002; Han and Lim 2004; Shon et al 2005; Zhong et al 2006). DMSO is an effective hydrogen bond acceptor by disrupting inter- and intramolecular starch-starch/water-starch hydrogen bonds and forming DMSO-starch hydrogen bonds (French 1984; Jackson 1991). McGrane et al (2004) used various hydrogen bond-forming and breaking agents to study the rheological properties of amylose gels. They reported that the use of intermolecular hydrogen bond-breaking agents such as urea reduced gel strength significantly, presumably by decreasing the intermolecular network formation between water and amylose. The objective of this study was to examine the role of hydrogen bonding in starch gelatinization by measuring and comparing the thermal and rheological parameters of two rice starches with different amylose contents during heating in aqueous urea solution of various concentrations.

### MATERIALS AND METHODS

#### Materials

Waxy rice was obtained from Sage V Foods, LLC (Los Angeles, CA); Wells, a long-grain rice cultivar, was obtained from the 2003 crop of the Rice Research and Extension Center, Stuttgart, Arkansas. Starch was isolated according to an alkali-steeping

method (Yang et al 1984). The amylose content of starch samples was determined by potentiometric titration (Schoch 1964). Urea was purchased from (ICN Biomecicals, Amora, OH) and concentrations of 0, 5, 10, 15, and 20% (w/v) were prepared as the solvent.

#### Gelatinization Properties

Gelatinization parameters, including enthalpy ( $\Delta H$ ), and onset ( $T_0$ ), peak ( $T_p$ ), and conclusion ( $T_c$ ) gelatinization temperatures, were assessed by differential scanning calorimetry (DSC) (Pyris-1, Perkin-Elmer, Norwalk, CT). Rice starch of  $\approx 4.0$  mg (db) was weighed into an aluminum DSC pan and 8  $\mu$ L of urea solution was added using a microsyringe. The pan was hermetically sealed and allowed to equilibrate for exactly 1 hr at room temperature before analysis. An empty pan was used as reference. The sample was heated from 25 to 120°C at 10°C/min.

#### Rheological Properties

Dynamic rheological parameters, including storage ( $G'$ ) and loss ( $G''$ ) moduli, were determined by applying a small oscillating stress using a rheometer (AR 2000, TA Instruments, New Castle, DE) equipped with a parallel-plate geometry (20 mm diameter). The gap size was controlled at 1.0 mm, and the stress and frequency were set at 11.5 Pa and 1 Hz, respectively. Rice starch (0.66 g) was added with 2.0 mL of urea solution to achieve a final starch concentration of 33% (w/v). The starch slurry was vortexed, and 0.320 mL of the well-mixed starch slurry was immediately transferred to the dynamic rheometer. The edge of the parallel plate was sealed with mineral oil to prevent water evaporation during heating. The starch slurry was heated from 25 to 95°C at 1.5°C/min.

#### Statistical Analysis

The data were analyzed with ANOVA using the general linear models (GLM) (SAS Institute, Cary, NC). The statistical model employed for data from rice starches with components of starch molecules variations was

$$Y_{jkl} = \mu + SC_j + UC_k + (A \times T)_{jk} + \epsilon_{jkl}$$

where  $Y$  = thermal and rheological properties,  $\mu$  = reference,  $SC$  = effect of components of starch molecules ( $j = 1-2$ ),  $UC$  = effect of urea concentration ( $k = 1-5$ ),  $SC \times UC$  = effect of components of starch molecules  $\times$  urea concentration, and  $\epsilon_{jkl}$  = residual variation. Significant interactions and main effects were compared using Fisher's protected LSD. The interaction effect was described as significant only when  $P < 0.05$ .

### RESULTS AND DISCUSSION

The amylose contents of waxy and Wells rice starch were 0 and 17.7% (db), respectively, using the potentiometric titration method. Wells rice starch was included in this study to examine the role of amylose in modifying rice starch gelatinization and rheological properties in the presence of different aqueous urea concentrations.

<sup>1</sup> Department of Food Science, University of Arkansas, Fayetteville, AR 72704.

<sup>2</sup> Current address: Department of Nutrition and Food Science, Fu-Jen University, 510 Chung Cheng Rd, Taipei, Taiwan.

<sup>3</sup> Corresponding author. Phone: 479-575-3871. Fax: 479-575-6936. E-mail: yjwang@uark.edu

Gelatinization properties of waxy and Wells rice starches at different urea concentrations in water (0–20%, w/v) are listed in Table I. Waxy rice starch displayed significantly lower  $T_o$ ,  $T_p$ ,  $T_c$ , and  $\Delta H$  values and a broader gelatinization range (GR) than Wells rice starch. Lai and Lii (2000) also observed a decrease in the GR values with increasing amylose content of rice starches. They proposed that the presence of amylose improved the homogeneity of crystallites in granules, which might lead to a narrower GR value and a larger  $\Delta H$  of Wells rice starch in the present study. When aqueous solution of urea was used as the solvent, an increase in urea concentration resulted in a decrease in  $T_o$ ,  $T_p$ ,  $T_c$ , and  $\Delta H$  and an increase in GR for both waxy and Wells rice starches. The decrease in  $T_p$ ,  $T_c$ , and  $\Delta H$  was more pronounced for waxy rice starch as evidenced by their different slope values and the significant ( $P < 0.05$ ) interaction of starch type  $\times$  urea concentration (Table II). Although the effective water concentration changed when urea was added, the two typical DSC endothermic transitions and the constant gelatinization temperature for the first endothermic transition in the presence of limited water were not observed for all samples (Biliaderis et al 1980), indicating the addition of urea up to 20% to water did not change the effective available water concentration for starch gelatinization.

DSC measures the disordering of the crystalline domains in starch granules during gelatinization (Eliasson and Gudmundsson 1996), and urea is a hydrogen bond-breaking agent. The shift to lower gelatinization temperatures with increasing urea concentration indicates the important contribution of hydrogen bonding to starch crystalline structure. The more pronounced change in the gelatinization properties of waxy rice starch with increasing urea concentration suggests the participation of amylose in starch crystalline domains. Amylose may be present in the crystalline lamellae and promote hydrogen bonding with amylopectin branch chains. The gelatinization temperature of starch could also be indirectly affected by the glass transition of the surrounding amorphous regions because the crystalline lamellae would not begin to melt until the surrounding amorphous regions were melted first (Dono-

van 1979; Donovan and Mapes 1980). The presence of amylose in the amorphous lamellae might increase the glass transition, which subsequently increases the gelatinization temperature of rice starches.

The effect of urea addition on the storage modulus ( $G'$ ) and loss modulus ( $G''$ ) of waxy and Wells rice starches are shown in Figs. 1 and 2, respectively. The temperature at which  $G'$  increases drastically and instantaneously is designated as  $T_G$  (Lii et al 1996a). The  $T_G$  values of waxy rice starch were significantly lower than those of Wells rice starch at different urea concentrations (Fig. 1), implying that the presence of amylose in rice starch increased the close packing of starch suspension systems (Eliasson 1986). Keetels et al (1996) attributed the initial increase in  $G'$  to the progressive swelling of starch from a fluid-like system with dispersed particles to a semifluid system with packed deformable particles. Continuation of heating above  $T_G$  led to the increase in  $G'$  and  $G''$  and ultimately the maximum values ( $G'_{max}$  and  $G''_{max}$ ) of both waxy and Wells rice starches. The increase in  $G'$  and  $G''$  of rice starches was attributed to the formation of a hydrogen-bonded network between water and starch molecules (Caesar 1950). The  $G'_{max}$  and  $G''_{max}$  of Wells rice starch were much higher than those of waxy rice starch. Lii et al (1996b) also observed higher  $G'$  values from an indica rice starch when compared with a waxy rice starch at different solid concentrations. After reaching the  $G'_{max}$  and  $G''_{max}$ , the  $G'$  and  $G''$  of rice starches decreased on further heating, presumably from the fragmentation of swollen granules (Lii et al 1995).

Table III lists the rheological properties of waxy and Wells rice starches in varying urea concentrations during heating, including  $T_G$ ,  $T_{G,max}$  (temperature at  $G'_{max}$ ),  $G'_{max}$ ,  $G'_{95}$  ( $G'$  at 95°C), and  $\tan\delta_{95}$  (ratio of  $G''$  to  $G'$  at 95°C). The  $T_G$  and  $T_{G,max}$  decreased with increasing urea concentration from 0 to 20% for both starches, and the decrease was more pronounced for waxy rice starch (Table II), suggesting that amylose played an important role in modifying the rheological properties of rice starches. The  $G'_{max}$  and  $G''_{max}$  of Wells rice starch increased with increasing urea

TABLE I  
Thermal Properties<sup>a</sup> of Waxy and Wells Rice Starches at Different Urea Concentrations

Rice Starch	Urea Conc (% w/v)	$T_o$ (°C)	$T_p$ (°C)	$T_c$ (°C)	GR (°C)	$\Delta H$ (J/g)
Waxy	0	64.9	71.7	81.2	16.3	12.5
	5	61.2	66.5	75.9	14.7	11.6
	10	56.8	63.2	73.2	16.4	10.6
	15	53.6	59.2	69.6	16.0	8.5
	20	48.9	55.1	65.9	17.0	5.8
Wells	0	75.2	79.3	84.3	9.1	13.1
	5	71.1	75.5	80.5	9.4	12.3
	10	67.8	72.3	78.2	10.4	10.9
	15	64.1	68.4	75.4	11.3	9.4
	20	60.1	64.2	71.5	11.4	8.0

<sup>a</sup> Onset, peak, and conclusion gelatinization temperatures ( $T_o$ ,  $T_p$ ,  $T_c$ , respectively); gelatinization range (GR =  $T_c - T_o$ ); gelatinization enthalpy ( $\Delta H$ ).

TABLE II  
Statistical Analysis of Thermal and Rheological Parameters of Rice Starches at Different Urea Concentrations<sup>a,b</sup>

Parameters	Waxy	Wells	P Value <sup>c</sup>
$T_o$	$y = -0.792x + 65$ ( $R^2 = 0.997$ )	$y = -0.744x + 75.1$ ( $R^2 = 0.999$ )	0.3392
$T_p$	$y = -0.81x + 71.24$ ( $R^2 = 0.996$ )	$y = -0.746x + 79.4$ ( $R^2 = 0.998$ )	0.0004
$T_c$	$y = -0.738x + 80.54$ ( $R^2 = 0.990$ )	$y = -0.614x + 84.12$ ( $R^2 = 0.992$ )	0.0035
$\Delta H$	$y = -0.33x + 13.1$ ( $R^2 = 0.943$ )	$y = -0.262x + 13.36$ ( $R^2 = 0.990$ )	0.0032
$T_G$	$y = -1.124x + 62.26$ ( $R^2 = 0.997$ )	$y = -1.02x + 74.78$ ( $R^2 = 0.9997$ )	0.0037
$T_{G,max}$	$y = -1.028x + 65.76$ ( $R^2 = 0.9996$ )	$y = -0.898x + 79.56$ ( $R^2 = 0.992$ )	0.0011
$G'_{max}$	$y = 20.72x + 1871.6$ ( $R^2 = 0.146$ )	$y = 179.58x + 4514$ ( $R^2 = 0.953$ )	<0.0001
$G'_{95}$	$y = -0.74x + 155.8$ ( $R^2 = 0.277$ )	$y = -53.36x + 1828.8$ ( $R^2 = 0.777$ )	<0.0001
$\tan\delta_{95}$	$y = 0.0014x + 0.222$ ( $R^2 = 0.943$ )	$y = 0.0014x + 0.14$ ( $R^2 = 0.533$ )	0.0032

<sup>a</sup> Abbreviations as in Tables I and III.

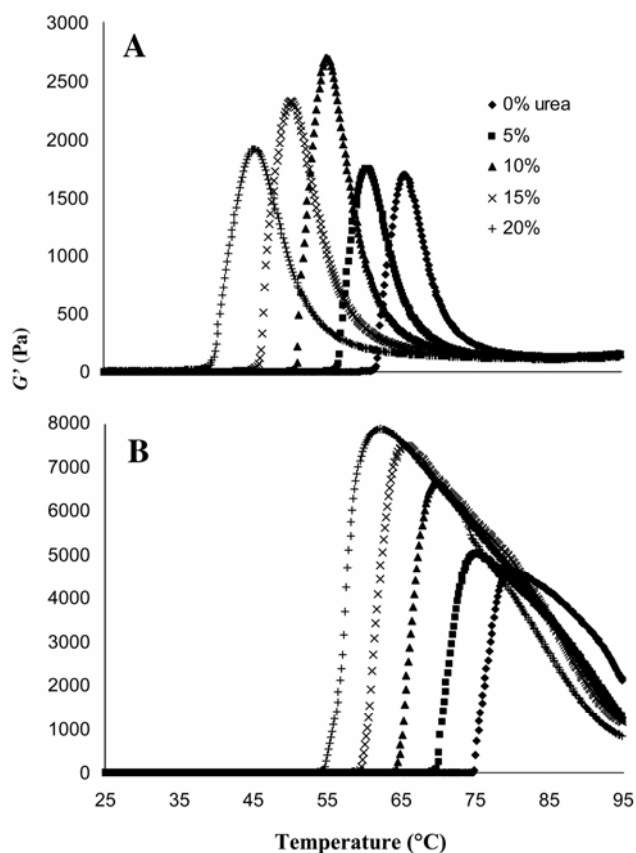
<sup>b</sup> Urea concentrations of 0, 5, 10, 15, and 20% (w/v) in water.

<sup>c</sup> Starch type  $\times$  urea concentration interaction.

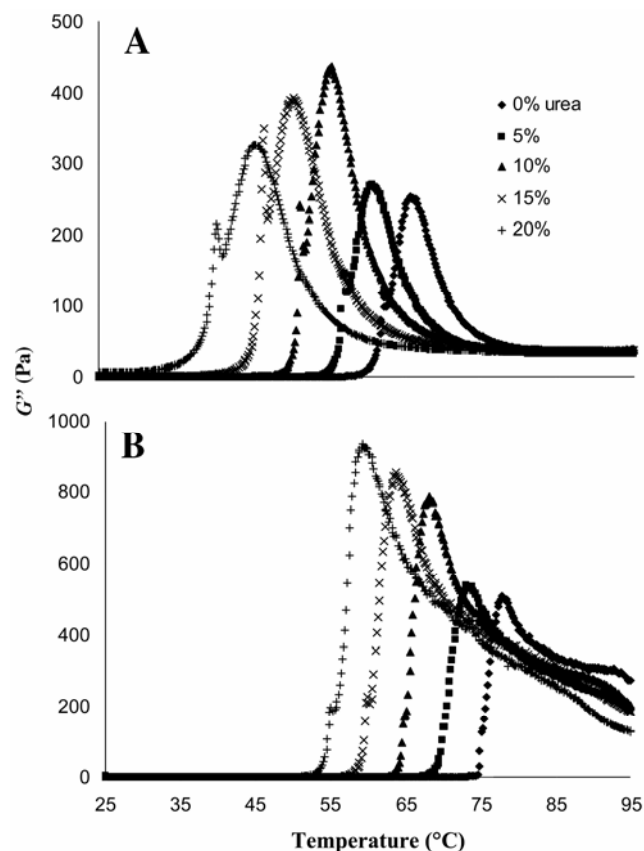
**TABLE III**  
Rheological Properties of Waxy and Wells Rice Starches at Different Urea Concentrations<sup>a</sup>

Rice Starch	Conc (% , w/v)	$T_{G'}$ (°C)	$T_{G'_{max}}$ (°C)	$G'_{max}$ (Pa)	$G'_{95}$ (Pa)	$\tan\delta_{95}$
Waxy	0	61.9	65.8	1,693	159	0.22
	5	56.8	60.7	1,748	139	0.23
	10	51.2	55.2	2,703	159	0.24
	15	46.0	50.5	2,330	150	0.24
	20	39.2	45.2	1,920	135	0.25
Wells	0	74.8	80.0	4,595	2,097	0.13
	5	69.8	75.2	5,008	1,231	0.15
	10	64.4	69.6	6,623	1,201	0.17
	15	59.4	65.9	7,469	1,137	0.16
	20	54.5	62.2	7,854	810	0.16

<sup>a</sup> Temperature at which  $G'$  increases drastically and instantaneously ( $T_{G'}$ ); temperature at which  $G'$  reaches maximum value ( $T_{G'_{max}}$ ); maximum value of  $G'$  ( $G'_{max}$ );  $G'$  at 95°C ( $G'_{95}$ );  $\tan\delta$  at 95°C ( $\tan\delta_{95}$ ).



**Fig. 1.** Storage modulus ( $G'$ ) of waxy (A) and Wells (B) rice starch (33%, w/v) in aqueous urea solutions at a heating rate of 1.5°C/min.



**Fig. 2.** Loss modulus ( $G''$ ) of waxy (A) and Wells (B) rice starch (33%, w/v) in aqueous urea solutions at a heating rate of 1.5°C/min.

concentration; however, those of waxy rice starch increased with increasing urea concentration from 0 to 10% but decreased when urea concentration was further increased to 20%. The interaction between amylose and amylose/amylopectin may contribute to the larger  $G'_{max}$  and  $G''_{max}$  of Wells rice starch and the continuing increase in  $G'$  and  $G''$  from 0 to 20% urea concentration for Wells. The decrease in  $G'_{max}$  and  $G''_{max}$  of waxy rice starch at urea concentrations of 15 and 20% may be the result of the strong hydrogen bond-breaking effect of urea that destabilized the starch-starch hydrogen bonds responsible for granular structure.

The  $\tan\delta_{95}$  is an index of viscoelastic property of starch at 95°C. The  $\tan\delta_{95}$  values of waxy rice starch were significantly higher than those of Wells rice starch (Table III). Wells rice starch showed higher  $G'_{max}$  and  $G'_{95}$  but lower  $\tan\delta_{95}$  than waxy rice starch, indicating that Wells rice starch exhibited more elastic characteristic than waxy rice starch during heating, presumably because of the presence of amylose. The  $\tan\delta_{95}$  of both waxy and Wells rice starches increased significantly with increasing urea

concentration, yet the effect of urea concentration on  $\tan\delta_{95}$  was more pronounced for waxy rice starch (Table II).

## CONCLUSIONS

The addition of urea altered the gelatinization and rheological properties of both waxy and Wells rice starches, and more pronounced changes were observed in waxy rice starch. The effect of urea addition on the gelatinization and rheological properties of rice starch varied with starch composition and urea concentrations.

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