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^{1}H NMR studies of starch–water interactions during microwave heating

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ABSTRACT

The aim of the present study was to investigate the effect of microwave heating on water distribution and dynamics in starch granules during the gelatinization of starch. Starch samples treated with microwave heating, rapid conventional heating and conventional heating was measured by ^{1}H NMR to examine the water distribution and dynamics in rice starch granules at a water activity of 0.686. The system proton longitudinal and transverse relaxation times were determined using inversion recovery (IR) and Carr–Purcell–Meiboom–Gill (CPMG) pulse sequences. The results showed that the T_1 of the water molecules in the samples treated with any of the three heating methods exhibited two distinct spectral peaks over the temperature range of 40–60 °C. With rising temperature, the long T_1 component and the short T_1 component approached each other, showing a trend of gradual convergence, while T_2 exhibited a single peak over the entire temperature range examined. In addition, significant differences were observed in the T_1 and T_2 of the water molecules in the samples heated by microwave, rapid conventional and conventional. The results show that the rapid heating effect of microwave inhibits the destruction of the hydrogen bonds between starch and water molecules. In contrast, the vibration motion of polar molecules caused by microwave heating accelerates the destruction of hydrogen bonds, producing a much stronger effect than the rapid heating effect of microwave.

1. Introduction

Conventional heating is driven by heat conduction and convection. The heat conduction occurring through heat transfer caused by the thermal motion of microscopic particles, such as molecules, atoms and free electrons (Xia & Chen, 2005). Heat convection is the transfer of heat from one place to another by the movement of fluids. Heat is transferred from the high-temperature portion to the low-temperature portion of a substance through the temperature difference between the two parts of the system, i.e., from the surface of an object to its interior, until a relatively uniform temperature distribution is achieved. Unlike conventional heating, microwave heating works through the alternating electric and magnetic fields of microwave radiation, which cause changes in the polarity orientation of dipole molecules in a substance. Under 2450 MHz of microwave radiation, polar molecules rotate up to billions of times per second, leading to rapid intermolecular friction, collision, vibration and the generation of heat. During this microscopic process, microwave energy is quickly converted to thermal energy in the medium to achieve heating without a thermal gradient, leading to a rapid increase in temperature (Sumnu, 2001).

Studies using a variety of detection methods to investigate the physicochemical properties of starch suspension systems heated by microwave and conventional heating have been extensively reported in the literature. Differential scanning calorimetry (DSC) has been used to compare the gelatinization properties of starch treated with microwave and conventional heating methods (Bilbao-Sáinz, Butler, Weaver, & Bent, 2007; Ndife, Sumnu, & BayIndIrlI., 1998; Palav & Seetharaman, 2007). X-ray diffraction (XRD) has been employed to examine changes in the crystallinity of starch (Bilbao-Sáinz et al., 2007; Lewandowicz, Jankowski, & Fornal, 2000) and alterations in the crystal structure of potato starch (Lewandowicz, Fornal, & Walkowski, 1997) following microwave heating. Optical detection methods such as scanning electron microscope (SEM), light microscopy and transmission

Abbreviations: RCV, rapid conventional heating; CV, conventional heating; MV, microwave heating.

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electron microscope (TEM) have been broadly utilized for the observation of morphological changes in starch granules treated with microwave and conventional heating methods (Bilbao-Sáinz et al., 2007; Palav & Seetharaman, 2006; Xue, Sakai, & Fukuoka, 2008). Some researchers have used rheological methods to examine the apparent viscosity and rheological properties of starch treated with microwave radiation and to quantify the kinetics of starch gelatinization (Anderson & Guraya, 2006; Lewandowicz et al., 1997, 2000). Zhang, Chen, Liu, & Wang (2010) employed Fourier-transform infrared spectroscopy (FT-IR) to study the short-range-order structure, e.g., chain structure and double-helical structure, of resistant canna starch pretreated with microwave heating.

Despite the research cited above, few reports on the dynamic state of the water content within starch granules during microwave treatment have been published. ^{1}H NMR is undoubtedly the most effective technique for the study of the dynamic behavior of water in a uniform system. Furthermore, it should be pointed out that to better interpret water protons relaxation data in greatly hydrated heterogeneous systems a complete and well-built model has been developed, based on the effect of diffusive and chemical exchange of water molecules system (Hills, Wright, & Belton, 1989; Hills, Takacs, & Belton, 1990). In fact, ^{1}H NMR has been extensively used to study the correlation between water proton relaxation processes and the random distribution of water in starch granules. Free induction decay (FID) and CPMG are the two most commonly used methods that have been employed in the detection of water distribution in different types of raw starch granules (Choi & Kerr, 2003a; Tang, Godward, & Hills, 2000). Choi and Kerr (2003b) used these two methods to study the effects of two chemical modifications, hydroxypropylation and cross-linking, on molecular motion of both starch and water in low-moisture wheat starch. Ritota, Gianferri, Bucci, and Brosio (2008) used CPMG imaging to examine the proton relaxation changes in a starch suspension system during expansion and gelatinization.

The present study expands the use of NMR technology to study of the microscopic distribution and dynamic state of the water in starch granules during microwave treatment. To eliminate the effect of the rapid heating characteristic of microwave heating, we established a rapid conventional heating model with the same heating rate as that of microwave heating for sample treatment. We used this model to investigate the effect of microwave heating on water distribution and dynamics within starch granules during gelatinization. The goal of this research was to elucidate the function of microwave vibration and rapid-heating mechanisms during the gelatinization of starch materials. Samples treated with conventional heating were included as controls.

2. Materials and methods

2.1. Materials

Starch was isolated from rice as described by Li (2008) with slight modification. Rice starch (protein content $\leq$0.23%, starch content $\geq$94.22%, amylose content of 15.11%, particle size of 5.52 ± 0.23 μm, enthalpy of 10.62 ± 0.22 J/g, peak temperature at 69.89 ± 0.13 °C) was isolated and purified from fresh rice (QiuShouBao Rice Products Co., Ltd, Anhui, China). The fresh rice (30 g) was steeped in distilled water (150 mL) for 18 h and grounded. The pH of the slurry was adjusted to 5.0 with 0.1 mol/L NaOH (Sinopharm Medicine Holding Co., Ltd., Shanghai, China), and 0.04 g cellulase (XueMei enzymic preparation Sci. Co., Ltd., Wuxi, China) was added to the slurry and reacted at 50 °C for 2 h. Then the pH was adjusted to 7.0 with 0.1 mol/L NaOH, and 0.9 g Neutral protease (Neutrase 0.8 Au/g, Novo Nordisk A/S, Denmark) was added and reacted at 50 °C for 4 h. The slurry was centrifuged at 10,000 × *g* for 30 min. The soft, top layer was carefully removed and the starch layer at the bottom was reslurried and washed with deionized water nine times. 100 mL 63% (v/w) n-butyl alcohol (Sinopharm Medicine Holding Co., Ltd., Shanghai, China) was added to the starch, stirred for 18 h to remove the fat and washed with deionized water for three times. The isolated starch was dried by lyophilization (Labconco Corporation, Kansas, USA), and then crushed by the Analysis grinding apparatus (IKA A11 basic, Guangzhou, China), and stored in a dryer at room temperature until analyzed.

2.2. Methods

2.2.1. Sample preparation

2.2.1.1. Conventional heating. The sample (6%) made by rice starch (6.00 g) and distilled water (9.00 g) was prepared in a quartz beaker. The slurry was heated on a hot plate to achieve slow heating; the heating was accompanied by agitation at a uniform speed to ensure even system heating. A fiber-optic probe was employed for online monitoring of the sample temperature. Samples were collected at specified temperatures. The collected samples were immediately placed in an ice bath to terminate the increase in sample temperature; they were then ready for use in subsequent experiments.

2.2.1.2. Rapid conventional heating. The sample (6%) made by rice starch (3.00 g) and distilled water (47.00 g) was prepared in a quartz beaker. The slurry was heated in an oil bath at 200 °C, a temperature determined by preliminary experiments, to achieve rapid heating. The remaining processing steps were the same as those used in conventional heating.

2.2.1.3. Microwave heating. Samples prepared as described in Section 2.2.1.2 were mixed well in a quartz container before being placed into the reaction chamber of a 2450-MHz microwave workstation (Xianou Instrument Manufacturing Co., Ltd., Nanjing, China). Program-controlled heating was carried out in four steps: (1) heating at 1200 W for 32 s, (2) heating at 600 W for 20 s, (3) heating at 300 W for 14 s and (4) heating at 600 W for 12 s. A fiber-optic probe was employed for online monitoring of the sample temperature during the process. The remaining processing steps were the same as those used in conventional heating.

2.2.2. NMR measurements

2.2.2.1. Sample preparation. Samples treated using the three heating methods were lyophilized in a lyophilizer (Labconco Co., Ltd., Kansas City, USA) and ground into particles smaller than 75 μm in a mill grinder (IKA Works Guangzhou, Guangzhou, China). Water equilibrium was achieved in the samples through incubation in a desiccator with a saturated $BaCl_2$ solution inside a 20 °C thermostatic chamber. The water activity was measured every 48 h using a HYGROLAB water activity meter (Rotronic Instrument Corp., New York, USA). The water activity of the samples was maintained at 0.686 without any further changes after 14 days.

Relaxation time was measured using a 22.96 MHz ^{1}H NMR spectrometer (NMI20-Analyst, Suzhou Niumai Electronic Technology Co., Ltd.). Equilibrated samples (approximately 0.5 g) were placed in an NMR tube with an outer diameter of 15 mm. The NMR tube was sealed with parafilm to prevent water loss. All procedures were carried out at 20 ± 0.1 °C. Each sample was measured in triplicate.

2.2.2.2. T_1 measurements. The longitudinal relaxation time T_1 was determined using the IR pulse sequence performed on a ^{1}H NMR spectrometer (Ernst, Bodenhausen, & Wokaun, 1987). The experimental parameters were as follows: the inversion time (TI) was 600 ms, the waiting time (TW) was 2 s, and eight scans were acquired for each measurement.

2.2.2.3. T_2 measurements. The transverse relaxation curve was determined using the CPMG pulse sequence performed on a ^{1}H NMR spectrometer (Meiboom & Gill, 1958). The experimental parameters were as follows: the echo time (TE) was 120 μs, the echo number was set to 1000, the waiting time (TW) was 2 s, and eight scans were acquired for each measurement.

2.2.3. Analysis of NMR relaxation date

2.2.3.1. Distributed exponential fitting (Micklander, Thybo, & van den Berg, 2008; Mortensen, Andersen, Engelsen, & Bertram, 2006; Ritota et al., 2008). The Longitudinal relaxation signal was fitted using a multi-exponential model. The mathematical expression of the relaxation signal in the longitudinal relaxation process is as follows:

$$M(\tau) = \sum_i M_{\infty(i)} \left[1 - 2\exp\left(\frac{-\tau}{T_{1(i)}}\right)\right] \quad (1)$$

where $M(\tau)$ is the signal amplitude at time τ and $M_{\infty(i)}$ and $T_{1(i)}$ are the signal percentages and the spin-lattice relaxation time, respectively, of component i.

The transverse relaxation signal was fitted using a multi-exponential model. The mathematical expression of the relaxation signal is as follows:

$$A(\tau) = \sum_i A_i \exp\left(\frac{-\tau}{T_{2(i)}}\right) + L_0. \quad (2)$$

where $A(\tau)$ is the echo amplitude at time τ, $A_{(i)}$ and $T_{2(i)}$ are the spin–spin relaxation amplitude and time, respectively, of component i, τ is the acquisition time axis, and the constant L_0 represents the decay curve noise. $T_{2(i)}$ provides a qualitative description of the relaxation and the position of water populations, the $A_{(i)}$ gives the quantitative description.

2.2.3.2. Principal component analysis (PCA) of the CPMG decay. PCA was performed on the T_2 relaxation decays, PCA is a statistical method used to find the main variations in the multivariate data set by creating new linear combinations from the underlying latent structures in the raw data. The systematic variation is described by the principal components (PC's), each representing the outer product of scores and loadings. The number of PCs required to describe the data was validated through full cross-validation, and only validated results were reported.

2.2.3.3. Calculation of the geometric mean of the T_2 spectrum. The geometric mean of the NMR T_2 spectrum (T_{2gm}) (Bryan, Wen, & Kantzas, 2002) was used to characterize T_2, an important parameter that reflects the dynamic state of the water content in a system. Assuming that a total of n time components are present, its calculation formula is as follows:

$$T_{2gm} = \left[\prod (T_{2i})^{P_i}\right]^{1/\sum P_i} \quad (i = 1, 2, 3 \ldots, n) \quad (3)$$

where P_i is the signal intensity of the T_2 spectrum corresponding to the time component i, T_{2i} (ms).

2.2.4. Statistical analysis

Multiple correlation coefficients (R^2) were calculated using Matlab 7.0 (The Mathworks Inc., USA). Data from three replicates per sample were subjected to analysis of variance (one-way ANOVA) using the LSD test (SPSS 9.0, SPSS Inc., USA) to determine the level of significance at $P \leq 0.01$.

A multivariate date analysis was performed for all raw T_2 relaxation curves. These curves were normalized by setting the largest sampled echo to a value of 1 and scaling the rest of the echo-train thereafter. The text files were imported to SAS 9.1 (SAS Institute, Cary, NC, USA) for principal component analysis (PCA) using an in-house written SAS macro. All data analysis and graph construction were performed with SAS/STAT and SAS/GRAPH.

3. Results and discussion

3.1. Comparison of the temperature curves obtained using three heating methods

Preliminary experiments generated highly coincident time–temperature curves for microwave and rapid conventional heating at sample temperatures below 65 °C. However, because microwave radiation, unlike conventional heating, has both electromagnetic and thermal effects, the heating rate of samples treated with the rapid conventional heating method gradually decreased after the samples reached 65 °C, while that of samples heated with microwave reached a plateau near 75 °C and then continued to rise at the previous heating rate. Because of this phenomenon, microwave heating with programmed temperature control (Section 2.2.1.3) was used to simulate rapid conventional heating to achieve the same heating rate for microwave heating as for each stage of rapid conventional heating. The function corrcoef in the data processing software Matlab 7.0 was used for correlation analysis. The multiple correlation coefficient, R^2, was 0.9989, indicating good fitness between the rapid conventional heating model and the microwave heating process. Thus, good simulation results were obtained. For comparison, samples were also treated through conventional heating. Time–temperature curves of the three heating methods are shown in Fig. 1. The average heating rates of microwave, rapid conventional and conventional heating were 1.5 °C/s, 1.5 °C/s and 0.05 °C/s, respectively.

3.2. Longitudinal relaxation from IR experiments

T_1 is the time required for an excited self-spinning proton to reach dynamic equilibrium after energy exchange with the surrounding lattice. It is measured through an IR sequence that reflects the energy relationship between the self-spinning proton and its environment and the status of the system in which the self-spinning proton resides (Zhang, 2007). In this study, by setting the echo wave interval to 120 μs, we filter the signal emanating from starch, so the hydrogen protons in the samples were almost derived from water. As shown in Fig. 2, samples treated with any of the three heating methods exhibited two distinct peaks distributed over the temperature range 40–60 °C, suggesting that the water molecules in the samples resided in two distinct regions. With rising temperature, the long T_1 component gradually evolved toward short relaxation times, while the short T_1 component approached long relaxation times, and the longitudinal relaxation time of the two components gradually converged.

The results presented in Table 1 show that the T_{11} of water in the relatively short T_1 region was distributed over the range 4–7 ms, which is due to hydrogen bonding of these water molecules with the surrounding starch. Because these water molecules were bound and restricted by the starch molecules, their movements were limited, resulting in a lower movement frequency close to the Larmor frequency and faster longitudinal relaxation, i.e., shorter T_1. Therefore, these water molecules are considered to be bound water. The T_{12} of water in the relatively long T_1 region was distributed over the range 24–76 ms. This fraction of the water was weakly restricted by the starch; thus, it is considered to be structured water.

From the results shown in Fig. 2 and Table 1, we concluded that the association of starch and water molecules through hydroxyl hydrogen bonds was the main process occurring in the samples treated with the rapid conventional and conventional heating methods before they reached 60 °C. In other words, the main

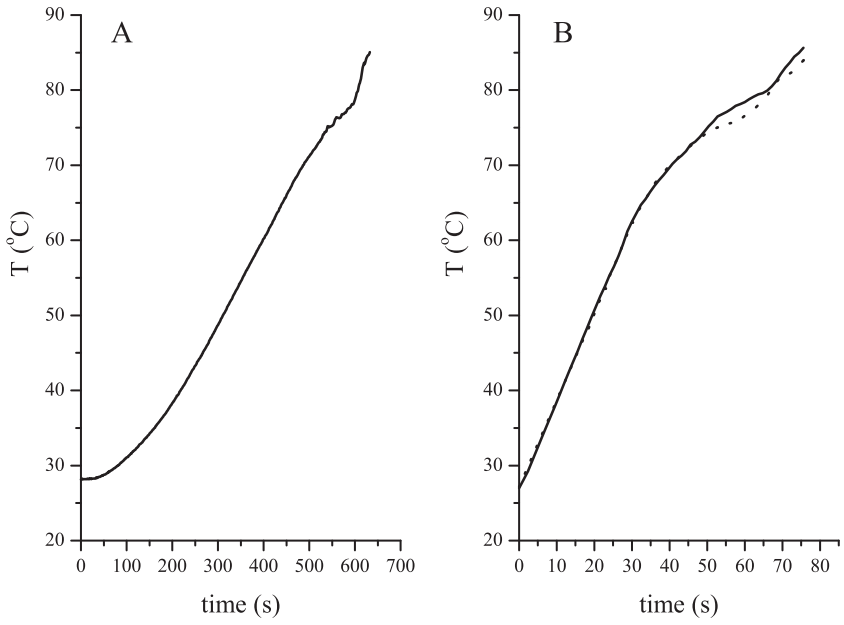

Fig. 1. (A) Temperature curves of the samples treated with conventional heating; (B) Temperature curves of the samples heated using microwave heating (—) and rapid conventional heating (—).

process occurring at this temperature was a water-absorption process. This process occurred in the samples heated with microwave before they reached 55 °C. The difference observed in the microwave-heated samples could be due to the microwave vibration mechanism, which may present certain obstacles to the association between starch and water molecules through the formation of hydroxyl hydrogen bonds. The T_{11} of the microwave-heated starch samples at 55 °C was larger than those of the samples heated with the other two methods, further indicating that the microwave-heated starch started to exhibit a weak water-binding capacity when reaching the gelatinization temperature. But we also can see that the differences of T_{11} between microwaved samples and the other samples were a little small, it is because the microwave energy is not too strong to destroy any bond directly. For the starch samples heated by the other two methods, the T_{11} of the samples subjected to conventional heating at 65 °C was higher than the T_{11} of samples submitted to rapid conventional heating. This result may have been caused by the longer heating time required for conventional heating. The slow heating permitted sufficient time for the starch to expand, which affected its restrictive capacity for water (Patel & Seetharaman, 2006). When the samples reached 55 °C through microwave heating or 60 °C through the other two heating methods, the starch slurry started to form hydrocolloid gels, in which the cross-links are usually composed of ordered 'junction zones'(Hansen et al., 2009). Then the 'junction zones' captured some water, which led to a reduction of the more mobile water fraction. So the mobility of the more mobile water is greatly reduced when a substance undergoes a transition from slurry to gel state, T_{11} and T_{12} exhibited apparent changes at this stage. Due to the further expansion of the starch, the structural barriers have been destroyed, leading to a faster exchange of water molecules between different regions as shown

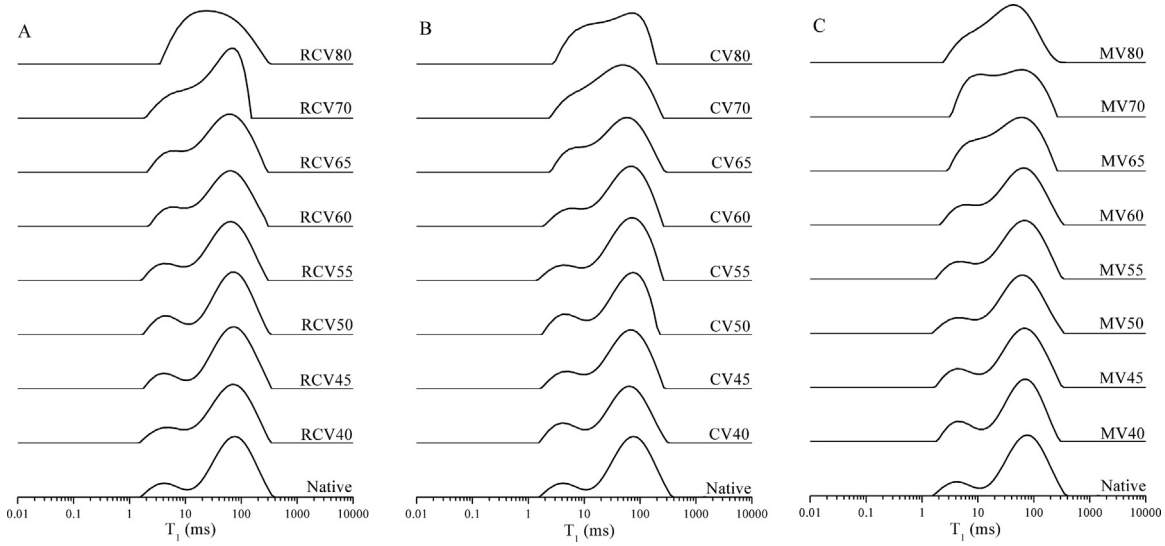

Fig. 2. The distribution of water proton longitudinal relaxation times (T_1) for starch samples treated with RCV, CV and MV at the indicated temperatures. (A, RCV; B, CV; C, MV).

Table 1
Proton longitudinal relaxation time values (T_1) of samples treated with different heating methods at different temperatures.

Temperature (°C)	RCV				CV				MV			
	$T_{1,1}$ (ms)	%	$T_{1,2}$ (ms)	%	$T_{1,1}$ (ms)	%	$T_{1,2}$ (ms)	%	$T_{1,1}$ (ms)	%	$T_{1,2}$ (ms)	%
40	4.1(0.2)B	20.1(0.5)	75.6(0.2)A	79.9(0.4)	4.0(0.1)C	22.2(0.1)	75.6(0.3)A	77.8(0.4)	4.0(0.1)C	22.1(0.3)	75.6(0.2)A	77.9(0.4)
45	4.0(0.2)B	18.3(0.6)	75.6(0.1)A	81.7(0.6)	4.1(0.2)C	21.1(0.2)	75.6(0.2)A	78.9(0.3)	4.0(0.1)C	21.3(0.3)	75.6(0.3)A	78.7(0.4)
50	4.0(0.2)B	21.1(0.8)	75.6(0.2)A	78.9(0.3)	4.0(0.2)C	22.1(0.1)	75.6(0.3)A	77.9(0.4)	4.0(0.2)C	20.3(0.2)	75.6(0.2)A	79.7(0.5)
55	4.0(0.1)B	21.2(0.6)	76.1(0.2)A	78.8(0.5)	4.0(0.1)C	19.3(0.1)	75.6(0.3)A	80.7(0.4)	5.3(0.2)B	24.2(0.4)	65.7(0.2)B	75.8(0.3)
60	5.3(0.2)A	26.4(0.4)	65.7(0.1)B	73.6(0.2)	5.3(0.2)B	23.3(0.2)	65.7(0.2)B	76.7(0.5)	7.0(0.1)A	27.4(0.3)	65.7(0.2)B	72.6(0.5)
65	5.4(0.2)A	27.2(0.3)	65.7(0.3)B	72.8(0.3)	7.0(0.2)A	26.4(0.2)	57.2(0.1)C	73.6(0.3)			57.2(0.3)C	98.4(0.5)
70			65.7(0.1)B	98.2(0.5)			49.8(0.2)D	99.2(0.2)			57.2(0.3)C	99.2(0.3)
75			57.2(0.2)C	99.2(0.4)			43.3(0.3)E	99.2(0.5)			49.8(0.1)D	99.2(0.4)
80			24.8(0.2)D	99.1(0.4)			34.4(0.3)F	99.3(0.5)			43.3(0.2)E	99.3(0.3)

All values shown are means (standard deviations) for three samples. Within each column, means followed by different letters (A, B, C, etc.) are significantly different ($P \leq 0.01$).

Table 2
Proton transverse relaxation time (T_2) of samples treated with different heating methods at different temperatures.

Temperature (°C)	RCV T_2 (ms)	CV T_2 (ms)	MV T_2 (ms)
40	1.0(0.08)C	1.0(0.09)C	1.2(0.09)D
45	1.0(0.11)C	1.0(0.06)C	1.2(0.08)D
50	1.0(0.09)C	1.0(0.08)C	1.2(0.09)D
55	1.2(0.07)C	1.2(0.10)C	1.3(0.07)D
60	1.2(0.12)C	1.3(0.11)C	1.8(0.11)C
65	2.0(0.11)B	2.3(0.11)B	2.3(0.12)B
70	3.0(0.07)A	2.7(0.07)A	2.7(0.09)A
75	3.0(0.07)A	2.7(0.06)A	2.7(0.08)A
80	3.0(0.09)A	2.7(0.09)A	3.0(0.07)A

All values shown are means (standard deviations) for three samples. Within each column, means followed by different superscripts letters (A, B, C, etc.) are significantly different ($P \leq 0.01$).

by the merging of the two peaks into one peak (Tananuwong & Reid, 2004). Therefore, T_{11} exhibited an increasing trend with decreasing T_{12}. Finally, due to the complete gelatinization of the starch, the starch granules ruptured, and the two types of water blended into a single water phase, which is represented by the merging of the T_{11} and T_{12} peaks into one peak in Fig. 2.

3.3. Transverse relaxation from CPMG experiments

To further study the relaxation behavior of the mobility components of starch, the present study utilized the CPMG pulse sequence to measure the transverse relaxation time. T_2 is the time required for an excited spin–spin proton to reach dynamic equilibrium after energy exchange with adjacent protons; it excludes effects due to the inhomogeneity of the surrounding magnetic field (Chen, 2007) and reflects the difference in the degrees of freedom of water. A higher degree of freedom is associated with longer transverse relaxation time, while a lower degree of freedom corresponds to a shorter transverse relaxation time (Pitombo & Lima, 2003). In the present study, the echo wave interval was set at 120 μs, and the signal emanating from starch was filtered (Choi & Kerr, 2003a). Therefore, the signals in the T_2 relaxation spectrum were mainly from the water content in the samples, with one peak distributed in the T_2 spectrum in Fig. 3 and T_2 distributed in the range of 1–3 ms. The presence of a single peak in the T_2 spectrum and double peaks in the T_1 spectrum was mainly due to the effect of diffusion on T_2. The rate of diffusion and chemical exchange of water molecules between two distinct regions is low compared to their intrinsic relaxation rates, which means that the time for the water content in the samples to diffuse across each region was far less than T_2 (Chatakanonda, Dickinson, & Chinachoti, 2003; Choi & Kerr, 2003a). Tang et al. (2000) obtained similar results and found that ^{1}H NMR can detect a single peak for bound water for A-type starch and the state of three types of water, corresponding to an amorphous growth ring, a semi-crystalline layer and channel water, for B-type starch. This result was determined to be due to the rapid chemical exchange of the water content in the starch granules due to the small size of A-type starch granules, which obscures the difference in the water content in different structural domains (Tang et al., 2000).

As shown in Table 2, with rising temperature, the T_2 of the samples heated by the three methods first stabilized to a specific value, followed by an abrupt elevation and eventually a stabilized level. This result indicated tighter binding between water and starch during the initial heating stage, with a smaller degree of freedom. With increased temperature, the starch rapidly folded, with a sharp rise in the degree of freedom of the water. Finally, starch granules ruptured due to gelatinization, leading to a stabilized interaction between water and starch.

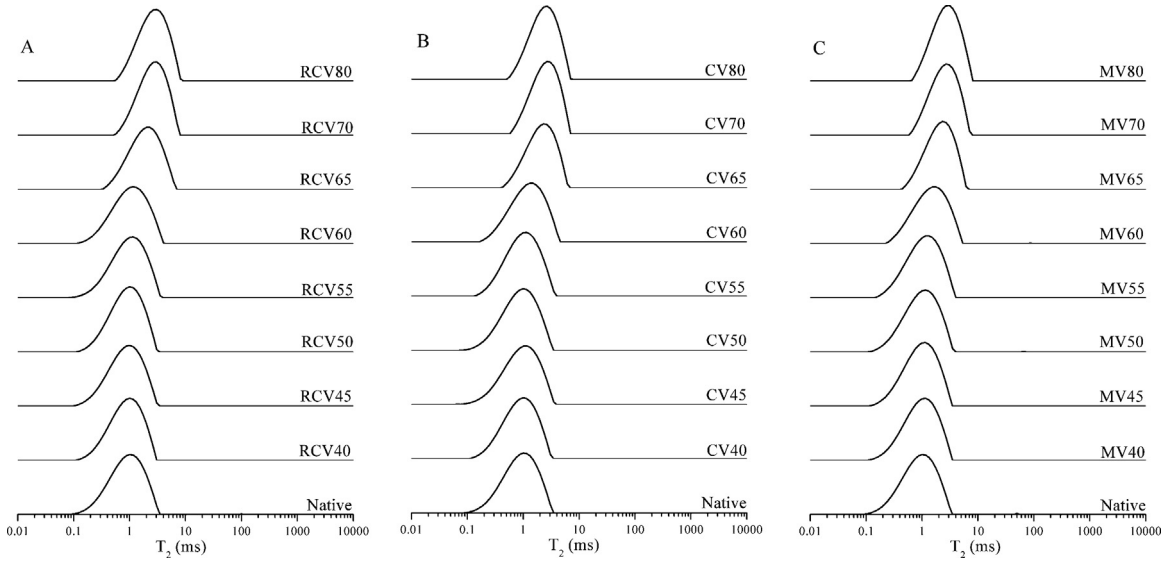

Fig. 3. The distribution of water proton transverse relaxation times (T_1) for starch samples treated with RCV, CV and MV at the indicated temperatures. (A, RCV; B, CV; C, MV).

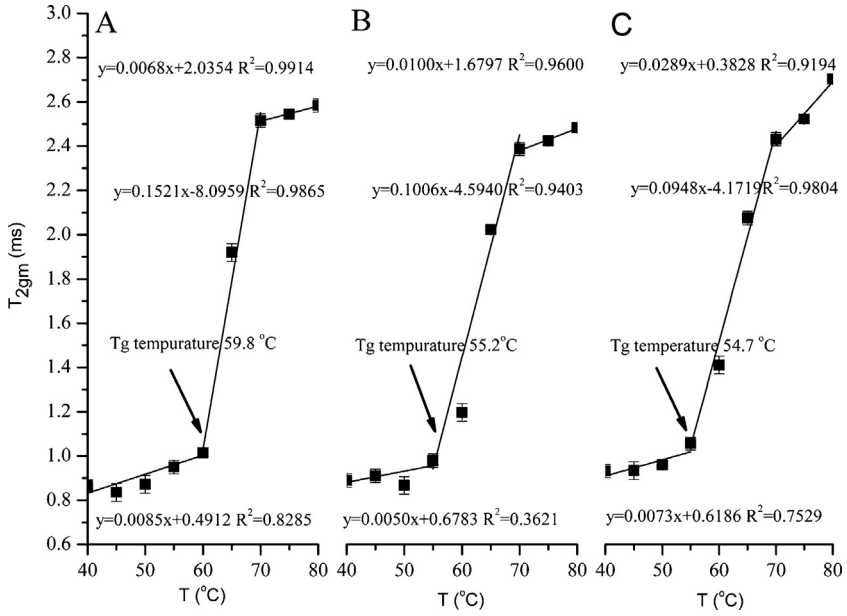

Fig. 4. NMR state diagram of samples heated by different methods. (A, RCV; B, CV; C, MV).

To compare the changes in T_2 in samples heated with the three methods, we used T_{2gm} to represent T_2 at each temperature point in the spectrum and plotted the change curves for the three sample groups. Clear inflection points were observed in the NMR state diagram as Fig. 4, and the intersection point of the two lines was calculated through linear regression; the corresponding temperature was the glass transition temperature of the samples (Pitombo & Lima, 2003; Zhang, 2007), as shown in Fig. 4. The glass transition temperatures of the samples heated with the three methods followed the descending order: rapid conventional heating, conventional heating and microwave heating. A small change in the chemical structure of starch can lead to a significant change in Tg. Although the crystalline region of starch does not participate in the glass transition, it limits the movement of the main chains of starch. Therefore, Tg increases with rising starch crystallinity (Wang & Chen, 2004), suggesting a smaller hydrogen bonds interaction inside the starch molecules of sample heated with microwave, with decreased rigidity and increased flexibility as reflected by a decrease in Tg. This effect may be due to the fast vibration of polar molecules caused by microwave heating. The Tg of conventional heated samples was smaller than that of rapid conventional heated samples. This result is likely due to the complete swelling of granules treated by conventional heating, resulting in a reduced effect from the hydrogen bonds inside the starch granules.

4. Conclusion

^{1}H NMR has been widely used to study the interactions between starch and water. By the analysis of proton relaxation curves different water populations in starch granules have been observed thus giving the possibility of a deeper insight into water dynamics and distribution in starch. In addition, data analysis based on a diffusive and chemical exchange model can quantify the relaxation

time, which provides very useful information for the analysis of the chemical exchange between starch and water. In this study, ^{1}H NMR technology was extended to the microwave heating of starch materials. With the use of the IR and CPMG pulse sequences, the proton relaxation curves were fitted using Eqs. (1) and (2). The analytical results showed that samples treated with rapid conventional heating possessed stronger hydrogen bonds between the starch and water molecules, leading to low water mobility. The strength of hydrogen bonds within samples treated with conventional heating was weaker, while samples heated with microwave had the weakest hydrogen bonds and the highest water mobility. The result can be explained that microwave heating involves both rapid heating and electromagnetic effects, and the rapid heating effect inhibited the destruction of hydrogen bonds between starch and water molecules, while the vibration motion of the polar molecules accelerated the destruction of hydrogen bonds. The effect of the vibration mechanism was significantly stronger than the rapid heating effect.

In short, ^{1}H NMR is shown here to be a simple and accurate method for the study of the water distribution and flow state in starch granules. To further address the role of the electromagnetic and rapid heating effects of microwave radiation in the gelatinization of starch, we will use small-angle X-ray scattering and ^{13}C NMR methods to comparatively analyze samples treated with microwave, rapid conventional and conventional heating to eventually provide a theoretical basis for improving the quality of microwave-reheated foods that contain starch.

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