Available online at www.sciencedirect.com
SCIENCE DIRECT
Polymer 47 (2006) 5888–5896
polymer
www.elsevier.com/locate/polymer

The phase composition and molecular motions of plasticized wheat gluten-based biodegradable polymer materials studied by solid-state NMR spectroscopy

Xiaoqing Zhang*, My Dieu Do, Pam Hoobin, Iko Burgar

CSIRO Manufacturing & Infrastructure Technology, Private Bag 33, Clayton South MDC, Clayton South, VIC 3169, Australia

Received 24 March 2006; received in revised form 15 May 2006; accepted 28 May 2006
Available online 15 June 2006

Abstract

The water plasticized wheat gluten (WG) materials were prepared by thermal processing and studied by dynamic mechanical analysis and solid-state NMR spectroscopy. The results indicate that the materials displayed a broad distribution of molecular motions and could be divided into different phases in terms of their mobility above the T_g. The rigid phase mainly consisted of proteins and starch with enhanced interactions between the two components via hydrogen bonding with water molecules. Lipid and water formed the mobile phase, however, lipid molecules were always more mobile than water. The intermediate phase consisted of plasticized starch and proteins (mainly proline and glutamine segments). The whole plasticized WG materials were heterogeneous at a scale of 20–30 nm, but the miscibility between proteins and starch was enhanced via increasing hydrogen bonding interactions with water molecules. Such strong hydrogen bonding acted as adhesion among these multi-components/phases over a wide range of temperature thus resulting in good mechanical properties of the materials. The results demonstrated that solid-state NMR techniques can provide valuable information of quantitative composition of phase structures with different mobility in a multi-component system and the chemical nature of each phase along with the interactions among these components/phases.

Keywords: Wheat gluten; Phase composition; Solid-state NMR

1. Introduction

Wheat gluten (WG) is one of the most important plant proteins with good viscoelastic properties, strong tensile strength and excellent gas barrier properties [1–3]. It has great potential to be used as a renewable and biodegradable natural polymer resource to apply in packaging, coating and binding applications [4–8]. Due to the strong self-association among wheat protein chains through intra-/intermolecular interactions and some extent of crosslinking via disulfide bonds among the polymer chains, thermal processing of wheat gluten is difficult. In addition, the properties of wheat proteins would be significantly modified on heating before melting, and the thermal decomposition plus crosslinking could all occur at higher temperatures [9–13]. Consequently a large amount of plasticizer is always required in thermal processing to reduce the strong intra-/intermolecular interactions among wheat protein molecules, increase the mobility of the protein chains, and therefore, improve the flexibility and the extensibility of the material [14–20]. Various chemicals such as water, glycerol, polyols, fatty acids and amines have been applied as plasticizing agents and their plasticizing effect on wheat gluten has been studied extensively [14,17–20].

Solid-state NMR has been demonstrated as a powerful technique to investigate plant protein systems, to explore the intermolecular interaction between protein molecules and plasticizers and to provide information on the molecular motion behaviour of each individual component in the multi-component system and phase structures of the whole plasticized protein-based materials [21–29]. In a recent paper [29], we have studied

* Corresponding author. Tel.: +61 395452653; fax: +61 395441128.
E-mail address: xiaoqing.zhang@csiro.au (X. Zhang).

0032-3861/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2006.05.060

several plasticized wheat protein systems using both water and glycerol as the plasticizer at scales from molecular level to tens of nanometers. Strong intermolecular hydrogen bonding interactions between the components in wheat proteins and the plasticizers resulted in a significant change in molecular motions, and the plasticizing effect was different in different wheat protein systems. Residual starch and lipid components also played different roles in the systems. Further detailed information was needed to quantitatively identify the phase composition of the systems and the chemical nature of these phases in terms of mobility.

A system of WG plasticized with water was studied in this paper. Water is the most ubiquitous plasticizer for wheat proteins because of its strong ability to modify the mobility of the protein molecules. Natural WG contains around 8–13% of moisture which is difficult to remove completely from the system. Thus, water always exists in the plasticized system at some level regardless of other plasticizer being present. Hydration of wheat proteins (including glutenins and gliadins) has been investigated comprehensively where solid-state NMR techniques were used to explore the hydration effect on different protein components and sub-units in order to provide chemical structure and dynamic information of the systems [26,27,30–32]. However, a relatively high amount of water/plasticizers was used in these hydration studies (usually over 30%) for food processing and solution casting. For development of wheat proteins-based biodegradable polymer materials via thermal processing, it is desirable to use a relatively lower level of plasticizers (10–30%). Most of those studies were focused on the mechanical and permeability performance of the materials without giving an insight into the plasticizing effect on intermolecular interactions and molecular motions of the materials and the plasticizing effect on different components in WG (e.g. proteins, starch and lipid) has not been understood in detail. In this paper, broad-line and high-resolution solid-state NMR techniques were applied to the water plasticized WG materials in order to obtain quantitative information of phase compositions in the thermally processed plasticized wheat gluten materials and chemical nature of these phases with different mobility.

2. Materials and methods

2.1. Materials

Wheat gluten (WG) vital, supplied by Manildra Group Australia, contained about 80% proteins, 15% residual starch, 4% lipid and less than 1% fibre and other impurities on dry basis. Sheet samples of the WG system were prepared using varied amount of water as plasticizer at pH = 4 as adjusted by acetic acid. A small amount (0.3% to WG) of Na_2SO_3 was added into the system in order to dissociate the disulfide bonding within the protein chains and to achieve efficient mixing among proteins and plasticizers [33,34]. Each sample with a designed amount of plasticizer was mixed with a high-speed mixer operated at a speed of 3000 rpm for 2 min, left overnight and then compression moulded at an optimum temperature of 130 °C for 5 min using a heating press with a pressure of 12 ton. The sample size was of 145 mm × 145 mm with a thickness of 1.0 mm ± 0.1 mm and stored in sealed plastic bags. As moisture loss could occur during thermal compression and testing at room temperature, all the experimental tests were conducted within 2–3 days after compression moulding and the moisture content in each sample was measured after drying the sample at 105 °C for 24 h and is listed in Table 1.

Table 1
Mechanical properties of the WG materials

Samples	Water content (wt%)	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)
W1	13.8	13.6	19.2	219.3
W2	15.7	7.5	57.4	143.0
W3	18.8	4.9	79.2	104.8
W4	21.2	3.0	91.4	67.5
W5	24.2	2.3	84.3	77.8

2.2. Instrumentation

DMA experiments were operated on a PerkinElmer PYRIS™ Diamond DMA in dual cantilevers bending mode at a frequency of 1 Hz. The temperature range was set at −100 °C to 150 °C and the heating rate was 2 °C/min. The storage modulus (E'), the loss modulus (E'') and tan δ (E''/E') were recorded as a function of temperature throughout the experiment.

Mechanical properties (tensile strength, elongation at break and Young's modulus) of the sheet samples were determined on an INSTRON 5566P with a crosshead speed of 50 mm/min. The data for each sample were obtained from an average of testing seven dog-bone specimens with an effective length of 30 mm and width of 6 mm.

Broad-line pulse solid-state ^{1}H NMR was carried out on a Bruker Minispec PC 120 spectrometer at 20 MHz. The 90° pulse was 4.5 μs with repetition of 2 s. The FID (free induction decay) signal of each sample was obtained by a solid-echo pulse sequence and a Carr–Purcel–Meiboom–Gill (CPMG: $90°x-(t_1-180°y-t_1\text{-echo})n$) pulse sequence at 40 °C. The 90°–180° pulse spacing (t_1) in the CPMG sequence was 50 μs, n was varied and totally 8 scans were used for each measurement. The whole FID of each sample was a combination of the data observed from solid-echo (time range of 0–200 μs) and from CPMG (time range of 400–2400 μs) pulse sequences, and then the FID was best fitted by multi-decay functions using IGOR program from WaveMetrics Inc.

High-resolution solid-state NMR experiments were conducted at room temperature using a Varian Unity plus spectrometer at resonance frequencies of 75 MHz for ^{13}C and 300 MHz for ^{1}H. ^{13}C NMR spectra were observed under either cross-polarization, magic angle spinning and high power dipolar decoupling (CP/MAS/DD) technique, or using a single 90° pulse excitation (SPE) method with high power decoupling. The 90° pulse was 4.5 μs for H-1 and C-13 while the spinning rate of MAS was set at a value in the range of 6–7 kHz. A contact time of 1.0 ms was used for measuring CP/MAS spectra while the repetition time was 2 s. The chemical shift of ^{13}C CP/MAS spectra was determined by taking the carbonyl carbon

of solid glycine (176.03 ppm) as an external reference standard. ^{1}H spin–spin (T_2) and spin-lattice (T_1) relaxation times were indirectly measured through ^{13}C resonances using the pulse sequences reported previously [35,36]. ^{1}H MAS NMR spectra were obtained with the same MAS rates and TMS was used as an external chemical shift reference. ^{1}H spin–spin (T_2) and spin-lattice (T_1) relaxation times were also measured directly through ^{1}H MAS spectra using the CPMG (t_1 of 40 μs) and the inversion recovery ($180^\circ - \tau - 90^\circ$) pulse sequences, respectively, with a repetition time of 2 s and a 90° pulse length of 2.5 μs.

3. Results and discussion

Plasticized wheat gluten materials usually present typical polymer properties and their performance strongly depends on the nature and the amount of plasticizer in the system. DMA was conducted for the plasticized WG system under the same conditions as reported previously [29] and the results are shown in Fig. 1. Note that the plasticized WG samples (W1–W5) displayed higher E' than that of WG at low temperature range. As the temperature increased, the onset of E' decreased and the tan δ peak associated with the glass transition (T_g) all shifted to lower temperatures when the amount of water increased. The tan δ peaks corresponding to T_g transitions of the plasticized samples were all very broad, and the maximum values of tan δ remained around 0.3 in all cases and lower than those observed in the WG system plasticized by both water and glycerol (around 0.5) [29], indicating a decrease in mobility of the WG system without glycerol. Minor onsets of E' decrease in conjunction with weak tan δ peaks at a temperature range around −40 to −70 °C were also observed corresponding to the β-transition of the plasticized WG materials. The β-transition became distinct as the amount of water increased which could be attributed to the glassy transition of bound water that was strongly hydrogen bonded with polymer chains in the system.

The T_g of the plasticized WG–water system strongly depends on the water content in the system, but it is normally difficult to detect due to the loss of moisture during the DSC or DMA measurement at high temperatures. In this paper, the T_g of the plasticized WG system is reported as the onset of the storage modulus E' decrease corresponding to T_g transition in DMA measurement. The T_g data of WG–water system are shown in Fig. 2, in conjunction with those with both glycerol and water as plasticizer taken from Ref. [29]. In the WG–water system the T_g decreased much faster than that in WG–water–glycerol materials with increasing amount of plasticizer, suggesting the plasticizing effect of water was more efficient than that of glycerol.

The mechanical properties of the WG materials are summarized in Table 1. When the amount of water content increased, tensile strength and Young's modulus of the samples decreased while the elongation of the materials significantly increased. As compared to the systems with both water and glycerol as plasticizer [29], the WG–water system exhibited weaker tensile strength and shorter elongation when a similar amount of plasticizer was used. This is consistent with the DMA results indicating water has a more efficient plasticizing effect as compared to glycerol. It also implies that the glycerol might have different interactions with gluten components under compression moulding conditions (130 °C/5 min) [37,38] that

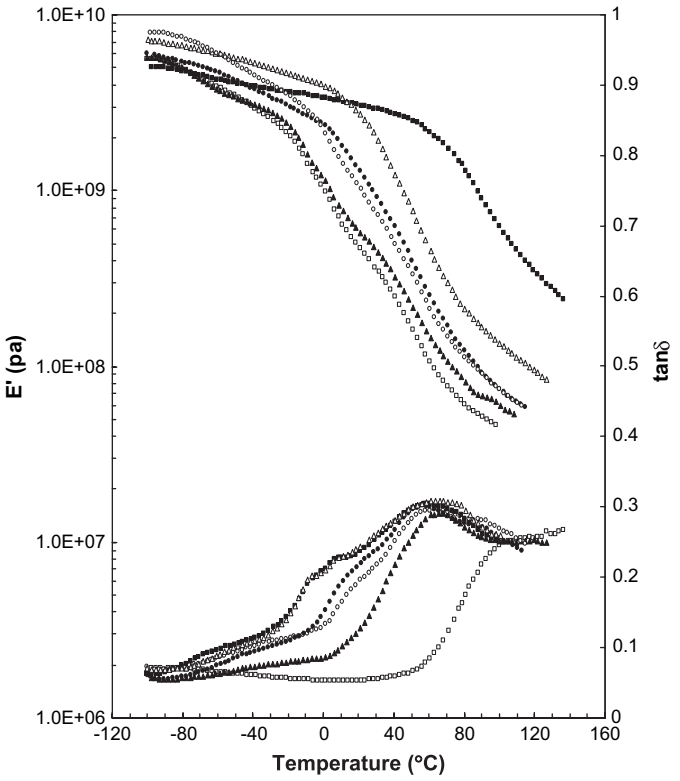

Fig. 1. The storage modulus (E') and tan δ of the WG samples from DMA. E' and tan δ of WG (■, □), W1 (△, ▲), W2 (●, ○), W3 (○, ●), W4 (▲, △) and W5 (□, ■).

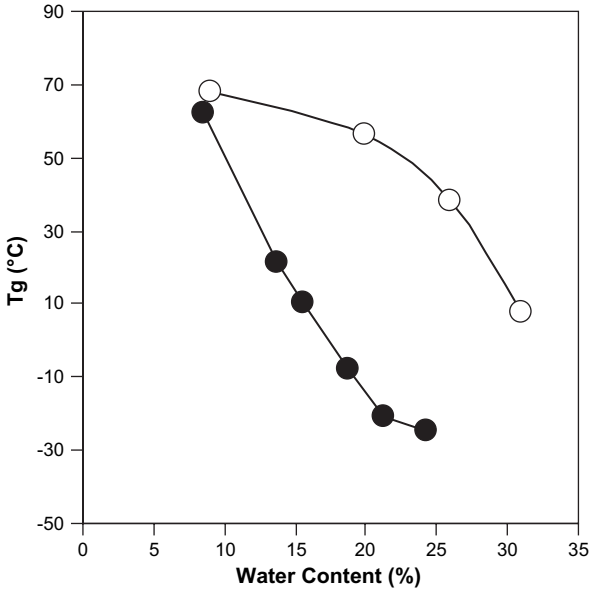

Fig. 2. The onset of the storage modulus E' decrease (corresponding to T_g transition) of the plasticized WG samples as a function of the plasticizer content of water (●) and water–glycerol (○).

stabilized the systems and contributed to the strength–elongation of the materials.

The main components in WG include a series of different proteins, starch and lipid. These components could interact differently with water molecules and show different plasticizing effects on molecular motions and finally display different phase structures. An understanding of the mobility behaviour of each component in such a complex system is fundamental for predicting the performance of a plasticized system to develop polymer materials with the desired properties. From this aspect, solid-state NMR provides a very powerful tool to obtain valuable information on phase structures, molecular motions of each component in a multi-component system and the interactions among these components by examining high-resolution NMR spectra in conjunction with various NMR relaxation parameters. These experiments can be conducted at room temperature ensuring that the samples retain the phase structures formed after thermal processing with a designed formulation.

Various molecular motions in a polymer due to different chemical structures, intra- and intermolecular interactions and varied phase morphologies would be reflected in NMR spectra and relaxation parameters [39]. ^{1}H spin–spin relaxation time (T_2) usually ranges from 10 μs for rigid polymers and to above several milliseconds for rubbery or viscoelastic polymers. Analysis of FID signals of a multi-component polymer system provides information of both mobility (the T_2 value) and proportion (the intensity of the T_2 component) of each component with different motional states and phase structures. Because the T_g of the WG–water system (W1–W5) varied from −25 to 20 °C and various phase structures with different mobility could co-exist in the system, the FID signals were obtained by a combination of the signals detected via both solid-echo (time scale of 0–200 μs) and CPMG (time scale of 400–2400 μs). The whole FID of W3 is shown in Fig. 3 as a typical example. Although, in general, there could be an infinite number of components in the system, three components were indeed clearly obtained by best fitting in all cases including a Gaussian decay with a short T_2 (T_{2S}) and two exponential decays with longer T_2 (T_{2M} and T_{2L}). This is somewhat different from those reported previously for hydrated wheat gluten [22,26]. In some previous reports, a T_2*, the time that FID decays to 1/*e* of its initial intensity, was used to characterize the hydration [24,30], but it mainly reflects the behaviour of components with a relatively short T_2 values. On the other hand, most of the previous FID data were obtained either by a single 90° pulse [22,24,26], where the dead time of a pulse could be a problem to detect the rigid components with T_2 around 10 μs while very mobile components could be missed, or by a solid-echo (or called quadrupolar echo) [27] which overcame the problem of dead time but could still experience difficulties for measuring very mobile components. The method to combine signals observed from both solid-echo and CPMG to obtain an FID used in this work should be a suitable way to detect all polymer components in a complex system with a wide range of ^{1}H T_2 values.

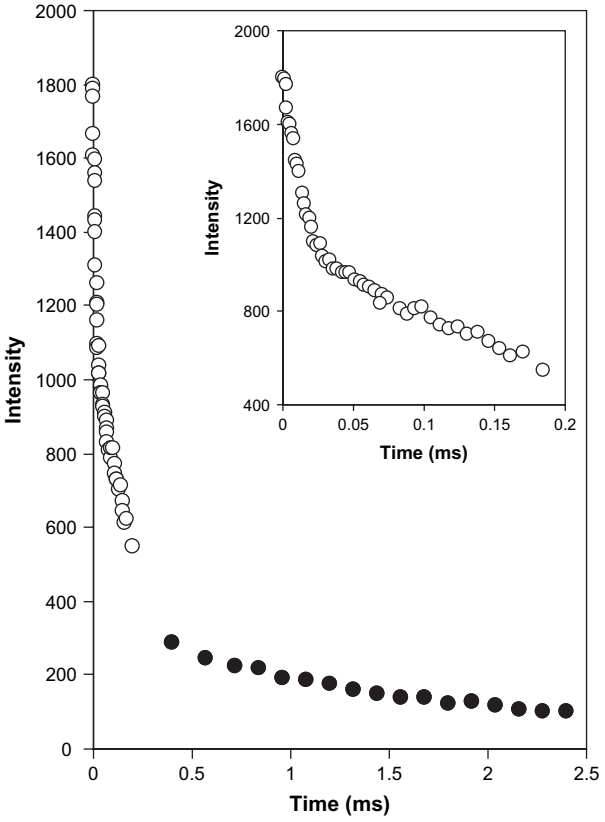

Fig. 3. The FID of W3 observed via a combination of signals observed by solid-echo (○) (with details shown in the inset) and CPMG (●) pulse sequences.

The ^{1}H T_2 data obtained from best fitting of FIDs for the plasticized WG system are summarized in Table 2. When no additional plasticizer was used, a short T_{2S} component (77.6%) was obtained for WG at around 9.5 μs corresponding to a rigid phase. There was only a very small amount (1%) of T_{2L} component (880 μs) in the system indicating that water as a plasticizer strongly interacted with WG molecules and appeared predominantly as bound water rather than as free water. An intermediate component was also observed with T_{2M} of 130 μs (21.3%). Note that the T_g of the WG sample was 65 °C (Fig. 2), but mobile components T_{2M} and T_{2L} were already present in the system at 40 °C. When the water content increased, the three T_2 components behaved differently. The T_{2S} proportion decreased as the amount of water increased, but the T_{2S} value only increased slightly. The T_{2M} values remained in the range of 150–160 μs for W1–W5 samples suggesting a constant mobility in the phase, but its proportion

Table 2
^{1}H T_2 values (μs) of the WG system observed from FIDs

Samples	T_{2S}	A_{2S} (^{1}H%)	T_{2M}	A_{2M} (^{1}H%)	T_{2L}	A_{2L} (^{1}H%)
WG	9.5	77.6	130	21.3	880	1.1
W1	10.7	50.5	161	43.5	1830	6.0
W2	10.7	46.6	150	37.8	1450	15.6
W3	12.1	39.8	151	43.7	1880	16.5
W4	12.9	30.2	158	54.6	2720	14.5
W5	12.3	23.4	147	61.0	3400	15.5

increased significantly as the water content increased. For T_{2L}, the proportion increased initially when the water content increased and then it remained constant around 15–16% but its T_{2L} value further increased significantly with increasing water content. Note that the T_g data of W1–W5 samples were all below room temperature (Fig. 2) while those of W3–W5 were even below 0 °C, however, rigid, intermediate and mobile components all co-existed in the system at 40 °C, indicating some components of WG still remained un-plasticized. The T_g of such a system was a temperature at which some proportion of polymer chains started to undergo glass transition. Based on the ^{1}H T_2 results, the phase composition of the WG–water system at 40 °C was constructed and shown in Fig. 4. It is interesting to note that when the amount of water increased to a certain level (e.g. 15 wt%), further increasing the amount of water decreased the proportion of the rigid phase, increased the amount of plasticized phase (T_{2M}) and enhanced the mobility of the T_{2L} phase, but the proportion of the T_{2L} remained constant. This suggested that the increased amount of water was mainly consumed in forming hydrogen bonding with protein and starch chains and mobilizing the rigid phase rather than remaining as free water in the system. This wide distribution of mobility in different phases contributed to a broad tan δ peak corresponding to the T_g transition as shown in Fig. 2. The presence of T_{2L} and increase of T_{2M} with increasing the water content did cause a decrease in tensile strength and modulus of the materials (Table 1), but the existence of the considerable amount of rigid phase resulted in the elongation remaining around 80–90% without further increasing with increasing water content. For such a multi-component/phase system, the phase structure/composition is more important for understanding the physical properties.

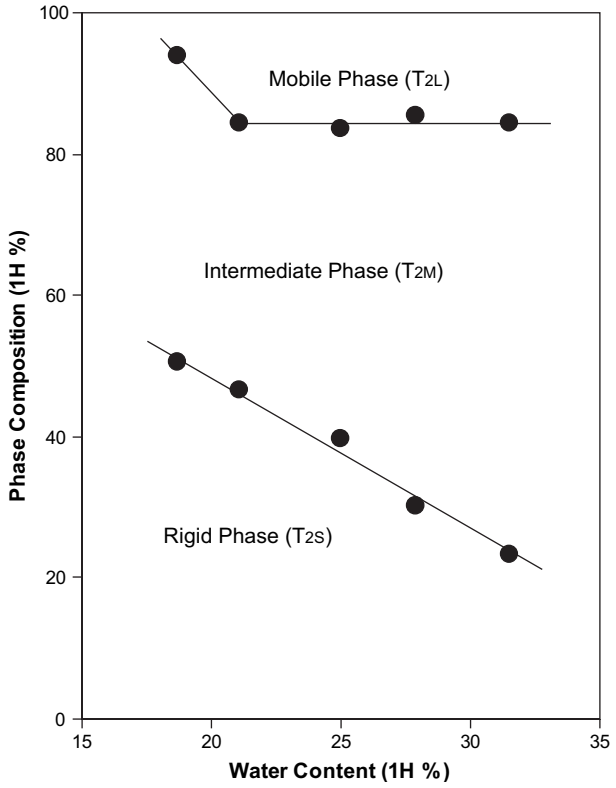

Fig. 4. The phase composition in mobility of WG–water system.

The CP/MAS/DD technique was applied to measure high-resolution solid-state ^{13}C NMR spectra of the WG–water system. The method is sensitive to the rigid materials when strong dipolar interactions in the system would efficiently enhance the polarization transfer from protons to nearby carbons thus enhancing the intensities of the carbon resonances. The CP/MAS ^{13}C NMR spectra of WG, W3 and W5 are shown in Fig. 5A. The major protein resonances at 173, 54, and 30–15 ppm are attributed to the carbonyl, C-α, C-β and C-γ carbons of proteins, respectively, while minor resonances in the spectrum at 157, 138, 129 and 116 ppm are due to Arg, Tyr and Phe [21,26,40]. The resonances of residual starch (~15%) were obtained at 103, 83 and 74 ppm (C-1, C-4 and C-2,3,5 of starch) while the C-6 peak at 62 ppm was overlapping with the resonances of the proteins. When varied amounts of water were applied to the system, no observable change was detected in chemical shift but the linewidth slightly increased when the amount of water increased in the system (W3 and W5).

In order to obtain signals of mobile components in the systems, the SPE method was used to observe ^{13}C NMR spectra with a repetition time as short as 2 s. The spectra of the same samples (WG, W3 and W5) are shown in Fig. 5B. These mobile components are mainly due to lipid (at 179, 130, 30–23 and 15 ppm) and a minor amount of mobile proteins (174, 61–45, 30–15 ppm) and starch (74 ppm). Plasticized samples W3 and W5 showed similar characteristics to that of WG, but the intensity of mobile proteins and starch slightly increased. The results shown in Fig. 5A and B indicated that the proteins and starch are the main components in the rigid phase while lipid is one of the main components in the mobile phase in conjunction with a small amount of plasticized mobile proteins and starch.

A model of ‘‘loop and train’’ was developed to describe the interaction behaviour in hydration of wheat proteins [1]. Glutamine played a key role in the dissociation of the hydrogen bonding between protein–protein interactions when increasing water content and in the formation of new hydrogen bonding between water and proteins in the hydration process [41]. It was relatively easier to form more mobile sections in both glutamine and glycine components [31]. More recent studies also suggested the involvement of proline in the hydration process [30]. The intensities at 61 and 52 ppm in SPE spectra (Fig. 5B) indicated the presence of proline (61 ppm) and glutamine (52 ppm) in the mobile phase and the intensity of glutamine became more pronounced when water content was higher (*ca.* W3 and W5).

High-resolution solid-state NMR technique also provides the possibility to conduct relaxation time measurements for each component in multi-component systems. As seen from the ^{13}C CP/MAS NMR spectra (Fig. 5A), the protein resonances at 173 ppm and 23–30 ppm and those of residual starch at 103 and 74 ppm are not overlapping with each other, therefore, the NMR relaxation parameters observed from these

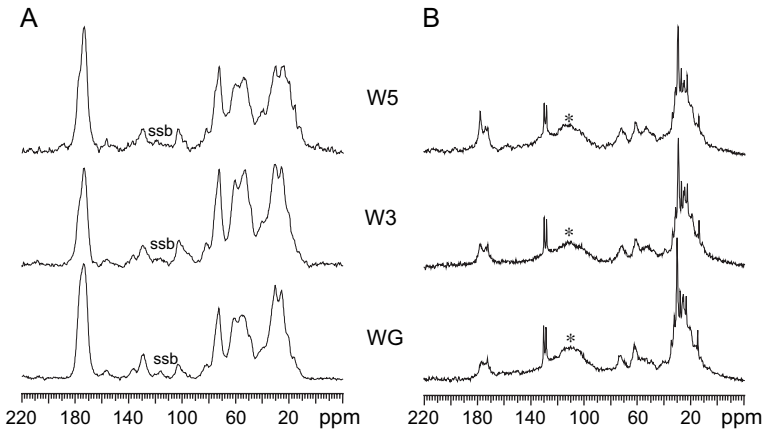

Fig. 5. ^{13}C CP/MAS (A) and SPE (B) NMR spectra of WG, W3 and W5 (ssb: spinning-side bands, * background signal of the spinner).

resonances should reflect the behaviour of wheat protein and starch components in the systems. The ^{13}C CP/MAS NMR spectra of W1 and W5 observed with varied CP delay times (τ) are shown in Fig. 6. After the 90° pulse in the proton channel, the proton resonances decreased their intensities following ^{1}H T_2 decay [36,42,43]. Because the intensities of ^{13}C resonances were enhanced by the proton magnetization through energy exchange during CP time, the intensities of each ^{13}C resonance of W1 or W5 observed vs. CP delay time reflected the ^{1}H T_2 decay of the rigid phases that were capable of transferring their magnetization to the ^{13}C resonance. Mainly the chemically bonded protons or those located close enough to the carbon in the structure can exchange energy with the ^{13}C resonance. Note in Fig. 6 that some resonances of proteins (174 and 54 ppm) and starch (74 ppm) were still present at $\tau = 50$ and 70 μs for W5, indicating the presence of relatively mobile proteins and starch components. The resonances at 25–30 ppm were attributed to both proteins and lipid. The ^{1}H T_2 data of the WG–water systems obtained via ^{13}C CP/MAS spectra with τ varied at a range of 2–90 μs are summarized in Table 3. Only the data of WG sample showed one Gaussian component for all resonances while ^{1}H T_2 data observed from each ^{13}C resonance of W1–W5 showed a combination of one Gaussian + one exponential decay. The T_2

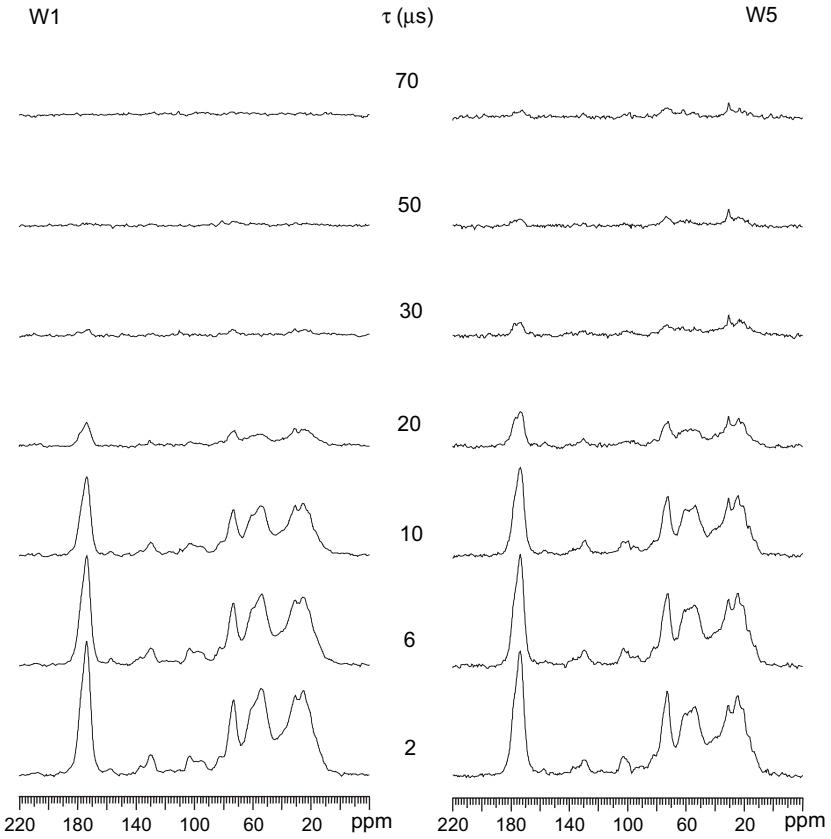

Fig. 6. ^{13}C CP/MAS NMR spectra of W1 and W5 measured with varied CP delay times (τ) where the intensity decay follows ^{1}H T_2 decay.

Table 3
^{1}H T_2 values (μs) of the WG system observed via ^{13}C CP/MAS spectra

Samples	174 ppm	74 ppm	54 ppm	30–25 ppm
WG	13.6	13.5	13.6	14.9
W1	11.9 (95%)	13.3 (92%)	12.8 (95%)	13.1 (94%)
	52 (5%)	50 (8%)	48 (5%)	57 (6%)
W2	12.0 (93%)	14.4 (89%)	13.0 (94%)	12.7 (91%)
	54 (7%)	56 (11%)	58 (6%)	71 (9%)
W3	12.1 (91%)	15.9 (89%)	13.9 (91%)	17.0 (90%)
	97 (9%)	95 (11%)	91 (9%)	96 (10%)
W4	16.5 (89%)	15.9 (88%)	14.7 (91%)	14.7 (79%)
	95 (11%)	103 (12%)	97 (9%)	106 (21%)
W5	16.7 (85%)	15.6 (85%)	15.4 (84%)	16.2 (69%)
	103 (15%)	109 (15%)	97 (16%)	98 (31%)

values of the Gaussian components observed via different resonances in each sample were also relatively identical. Their T_2 values increased slightly from 12–13 μs to 16–17 μs when water content increased corresponding to the T_{2S} component in Table 2. Meanwhile, the T_2 values of the exponential components observed via different resonances of each sample increased initially as the water content increased and then remained at a level of 100 μs (W3, W4 and W5) while the proportion continued to rise as water content increased. This phenomenon was similar to that of T_{2M} shown in Table 2, further confirming the presence of mobile protein and starch components in the intermediate phase. The difference in ^{1}H T_2 obtained from the CP/MAS and broad-line NMR methods is due to the different mechanism of the two techniques. The CP/MAS method is sensitive to the rigid polymer components thus only T_{2S} and T_{2M} components could be detected. The MAS could also average out some of the weak dipolar interactions. In addition, the CP/MAS NMR spectra were measured at room temperature (22 °C), while the broad-line FID signals were detected at 40 °C at which a longer T_2 value could be obtained for the mobile components.

The existence of mobile components in the system has made it possible to observe high-resolution solid-state ^{1}H NMR spectra by the MAS technique. Fig. 7 shows the ^{1}H spectra of W3 observed by CPMG pulse sequence using some typical τ times at which the nth echo appeared under static (A) and MAS (B) conditions as an example of the WG–water system. The peaks at 0.9, 1.3, 2.0, 2.7 and 5.3 ppm were all assigned to lipid resonances while the broad peak at 4.7 ppm was due to water. The minor amount of resonances of proteins (if existed) might

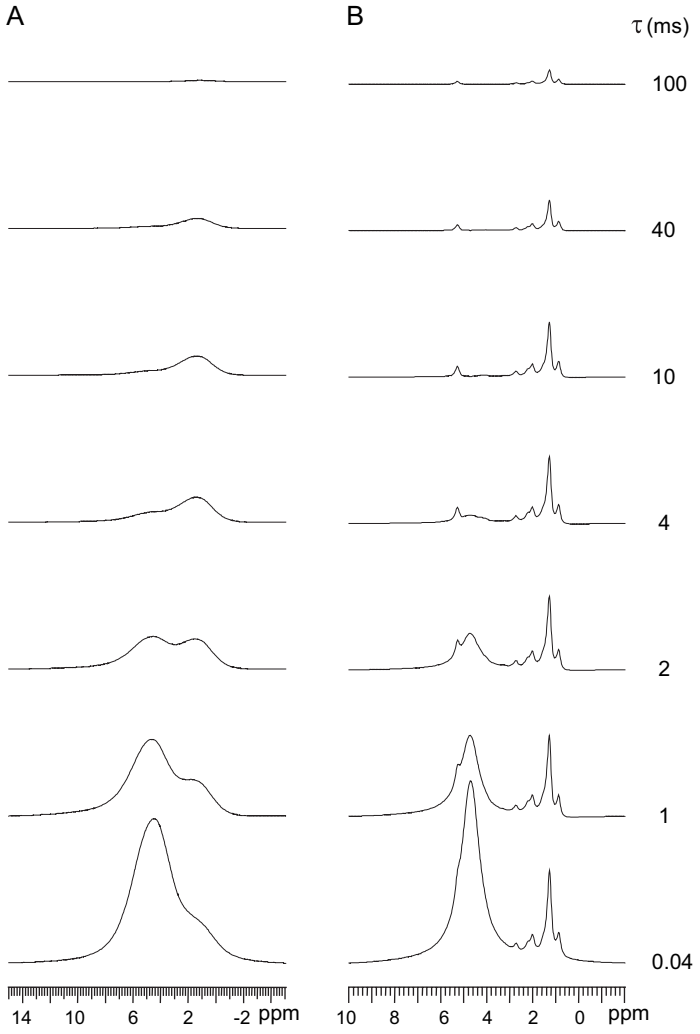

Fig. 7. ^{1}H NMR spectra of W3 observed by CPMG pulse sequence without MAS (A) and with MAS (B) under varied τ delay times.

Table 4
^{1}H T_2 values (ms) of the WG system observed via ^{1}H NMR spectra

Samples	MAS condition					No MAS	
	4.7 ppm	2.7 ppm	2.1 ppm	1.3 ppm	0.9 ppm	4.6 ppm	1.7 ppm
WG	0.23	2.83	10.0	22.5	13.4	–	7.1
W1	0.55	2.05	6.82	16.7	10.1	0.57	4.8
W2	0.79	1.84	7.96	21.5	20.4	0.71	4.0
W3	1.19	2.65	10.0	23.6	20.6	1.02	4.7
W4	1.38	3.22	10.6	20.6	19.1	1.25	4.4
W5	1.70	1.78	7.91	19.3	23.0	1.47	4.2

Table 5
^{1}H T_1 values (s) of the WG system

Samples	Via ^{13}C CP/MAS spectra				Via ^{1}H MAS spectra			
	174 ppm	74 ppm	54 ppm	30–25 ppm	4.7 ppm	2.1 ppm	1.3 ppm	0.9 ppm
WG	0.70	0.86	0.58	0.60	0.70	0.58	0.53	0.67
W1	0.58	0.38	0.31	0.32	0.46	0.48	0.46	0.52
W2	0.29	0.31	0.28	0.29	0.36	0.39	0.42	0.48
W3	0.35	0.33	0.36	0.31	0.28	0.35	0.41	0.47
W4	0.38	0.37	0.37	0.38	0.23	0.31	0.37	0.41
W5	0.38	0.38	0.39	0.39	0.25	0.28	0.34	0.37

be overlapping with the broad water peak in the range of 3.5–4.5 ppm. As the τ time increased, the intensity of the water peak decayed much faster than the lipid resonances under both static and MAS conditions, indicating lipid was more mobile than water in the system and always contributed to the T_{2L} phase. The application of MAS did increase the T_2 value (2–4 times) of lipid resonances and different resonances also displayed different T_2 values (shown in Table 4). The chain $-CH_2-$ (1.3 ppm) resonances always had the longest T_2 values while the resonances of $-CH_2-COO-$ (2.1 ppm) and $-CH=CH-CH_2-$ (2.7 ppm) had short T_2 values. No significant water effect to the data was obtained possibly due to the hydrophobicity of lipid. For water at 4.7 ppm, the T_2 value increased as the water content increased from 0.23 ms (WG) to 1.7 ms (W5), but the MAS only slightly increased the T_2 of water which is quite different from that of lipid. Previous papers [26,30,31] had reported that the ^{1}H linewidth of proteins was reduced significantly by increasing the speed of MAS but the linewidth of the water peak had minor change under the same conditions. The significant increase of T_2 of lipid under MAS condition was similar to those of proteins indicating an involvement of the homogeneous linewidth broadening mechanism. The linewidth broadening nature of water could be a result of chemical exchange between sites with different chemical shifts or relaxation processes. In a hydrated polymer system, different types of water that could co-exist are variously described as *bound* and *free*, or *non-freezable* and *freezable*, or *associated* and *free*, which may be attributed to the water molecules strongly bonded with polymer chains, and those having no interaction with polymer at all [39]. In most cases there is a wide distribution of water between the two extreme cases. The linewidth broadening nature of water indicated a broad distribution of different interaction types of water undergoing chemical exchange among these species. The short T_2 values of water (0.23–1.7 ms, not 10 ms–1 s) suggested there was little so-called *free* water in the system even when the water content was 24% (W5).

The study of ^{1}H spin-lattice T_1 relaxations of each component in a multi-components system provides not only the information of molecular motions at MHz range but also the homo- or heterogeneity of the system on a scale of *ca*. 20–30 nm, which is the effective spin-diffusion length during the T_1 relaxation times [42–46]. ^{1}H T_1 data of the plasticized WG system were observed either via ^{13}C resonances through CP/MAS (mainly the rigid phase/component) or via their ^{1}H MAS spectra (the mobile component/phase) and shown in Table 5. The comparison of ^{1}H T_1 data observed by the two methods should therefore provide the strength of dipolar interactions among different components or phases with different chemical nature and molecular mobility, thus exploring the homogeneity or heterogeneity of the whole system on a scale of 20–30 nm.

The ^{1}H T_1 data of protein components observed at 54 ppm and 30–25 ppm (via ^{13}C CP/MAS spectra) were quite similar in most of the plasticized samples indicating a strong spin-diffusion interaction. The ^{1}H T_1 data detected at 74 ppm reflected the behaviour of the starch component. Its value of 0.86 s in the WG sample was quite different from those of proteins, indicating the starch was not mixed intimately with the protein molecules at the scale of 20–30 nm. However, when the water content increased, the value observed for the starch component at 74 ppm decreased quickly and tended to be identical to those observed from protein resonances. This indicates that an increase in water content allows more water molecules to enter the rigid protein and starch phase, resulting in strong interactions among protein, starch and water molecules, generating an intimate mixing. Strong hydrogen bonding interactions and possible chemical exchange between water and starch/proteins played a key role in this process. The data observed through ^{1}H MAS spectra mainly reflected the behaviour of lipid (at 2.1, 1.3 and 0.9 ppm) and water (at 4.7 ppm). Each resonance displayed its own relaxation behaviour in the same sample (Table 5), suggesting weak spin-diffusion interactions among the lipid segments due to the high mobility of these components. The values decreased as water content increased, displaying a similar trend to that of water at 4.7 ppm. Comparison of the T_1 data observed from the two different methods (^{13}C CP/MAS and ^{1}H MAS) indicated that the plasticized WG materials were heterogenous at a scale of 20–30 nm, although an increase of water content did enhance the homogeneity between starch and proteins.

4. Conclusions

Water as a very efficient plasticizer for wheat gluten can enhance molecular motions in different components in the WG system. Such plasticized WG materials exhibited a wide distribution of molecular mobility and, accordingly, could be divided into three phases in terms of mobility at a temperature above the T_g. The rigid phase mainly consisted of proteins and starch with enhanced interactions among the two components with water. The proportion of rigid phase decreased as

the water content increased, but this phase still remained in the materials even when the water content was 24%. Lipid molecules were always the most mobile component in the system. The intermediate phase consisted of plasticized proteins (mainly proline and glutamine segments) and starch, while water and some lipid also contributed to this phase when water content was low. Increasing water content increased the proportion of this plasticized phase, but its mobility remained constant. The mobility of water molecules displayed a broad distribution in the system with strong dependence on water content. When water content was low, the water molecules were strongly hydrogen bonded with proteins and starch to form an intermediate phase with these plasticized protein and starch segments. An increase of water content also resulted in an increase of the amount of mobile water species which were assigned to the mobile phases. However, in all cases, there was little so-called ''free water'' in the system. The whole plasticized WG materials were heterogeneous at a scale of 20–30 nm. The phase structure/composition is more important for understanding physical properties of such multi-component/phase materials with a broad distribution of phase mobility over a broad temperature range. Strong hydrogen bonding acted as adhesion among different components/phases thus providing good mechanical properties of the materials over a wide range of temperature. The results have further demonstrated that the application of both broad-line and high-resolution solid-state NMR can provide valuable information on quantitative composition of phase structure and different mobility in a multi-component system, the chemical nature of each phase, and the interactions among these components/phases. The phase composition model obtained for WG–water system could be considered as a general picture for most plasticized protein materials.

References

[1] Belton P. Journal of Cereal Science 1999;29:103.
[2] Ornebro J, Nylander T, Eliasson A. Journal of Cereal Science 2000;31:195.
[3] Singh H, MacRitchie F. Journal of Cereal Science 2001;33:231.
[4] Scott G. Degradable polymers: principles and applications. 2nd ed. Dordrecht: Kluwer Academic Publishers; 2002.
[5] Kersting H, Lindhauer MG, Bergthaller W. Industrial Crops and Products 1994;3:121.
[6] Derksen JTP, Cuperus FP, Kolster P. Industrial Crops and Products 1995;3:225.
[7] Derksen JTP, Cuperus FP, Kolster P. Progress in Organic Coatings 1996;27:45.
[8] Weegels P, Verhoek J, Groot A, Hamer R. Journal of Cereal Science 1994;19. p. 31 and 39.
[9] Apichartsrangkoon A, Ledward D, Bell A, Brennan J. Food Chemistry 1998;63:215.
[10] Graaf L. Journal of Biotechnology 2000;79:299.
[11] Elizalde BE, Pilosof AMR. Journal of Food Engineering 1999;42:97.
[12] Hargreaves J, Popineau Y, Meste M, Hemminga M. FEBS Letters 1995;372:103.
[13] Micard V, Guilbert S. International Journal of Biological Macromolecules 2000;27:229.
[14] Pouplin M, Redl A, Gontard N. Journal of Agricultural Food Chemistry 1999;47:538.
[15] Irissin-Mangata J, Bauduin G, Boutevin B, Gontard N. European Polymer Journal 2001;37:1533.
[16] Pommet M, Redl A, Morel MH, Guilbert S. Polymer 2003;44:115.
[17] Redl A, Morel M, Bonicel J, Vergnes B, Guibert S. Cereal Chemistry 1999;76:361.
[18] Redl A, Morel M, Bonicel J, Guibert S, Vergnes B. Rheologica Acta 1999;38:311.
[19] Zhang X, Burgar I, Do M, Lourbakos E, Beh H. Polymer Preprints 2003;44(1):402.
[20] Pommet M, Redl A, Guibert S, Morel M-M. Journal of Cereal Science 2005;42:81.
[21] Belton PS, Duce S, Colquhoun IJ, Tatham AS. Magnetic Resonance in Chemistry 1988;26:245.
[22] Abelett S, Barnes DJ, Davies AP, Ingman SJ, Patient DW. Journal of Cereal Science 1988;7:11.
[23] Belton PS, Duce S, Tatham AS. Journal of Cereal Science 1988;7:113.
[24] Belton PS, Colquhoun IJ, Grant A, Weliner N, Field JM, Shewry PR, et al. International Journal of Biological Macromolecules 1995;17:74.
[25] Umbach SL, Davis EA, Gordon J. Journal of Cereal Science 1998;28:233.
[26] Calucci L, Forte C, Galleschi L, Geppi M, Ghiringhelli S. International Journal of Biological Macromolecules 2003;32:179.
[27] Calucci L, Galleschi L, Geppi M, Molloca G. Biomacromolecules 2004;5:1536.
[28] Zhang X, Burgar I, Lourbakos E, Beh H. Polymer 2004;45:3305.
[29] Zhang X, Burgar I, Do M, Lourbakos E. Biomacromolecules 2005;6:1661.
[30] Belton PS, Gil AM, Grant A, Alberti E, Tatham AS. Spectrochimica Acta Part A 1998;54:955.
[31] Alberti E, Gilbert SM, Tatham AS, Shewry PR, Gil AM. Biopolymers 2001;58:33.
[32] Alberti E, Humpfer E, Spraul M, Gilbert SM, Tatham AS, Shewry PR, et al. Biopolymers 2002;67:487.
[33] Gontard N, Guilbert S, Cuq JL. Journal of Food Science 1992;57:190.
[34] Micard V, Morel M-H, Bonicel J, Guilbert S. Polymer 2001;42:477.
[35] Alexlson DE, Russell K. Progress in Polymer Science 1985;11:221.
[36] Zhang X, Takegoshi K, Hikichi K. Macromolecules 1992;25:2336.
[37] Kokini J, Cocero A, Madeka H, de Graaf E. Trends in Food Science and Technology 1994;5:281.
[38] Madeka H, Kokini J. Journal of Food Engineering 1994;22:241.
[39] McBrierty V, Packer K. Nuclear magnetic resonance in solid polymers. Cambridge, UK: Cambridge University Press; 1993.
[40] Yoshimizu H, Ando I. Macromolecules 1990;23:2908.
[41] Shewry PR, Poplineau Y, Lafiandra D, Belton P. Trends in Food Science and Technology 2001;11:433.
[42] Havenes JR, VanderHart DL. Macromolecules 1985;18:1663.
[43] Dickinson LC, Yang H, Chu C-W, Stein RS, Chien JCW. Macromolecules 1987;20:1757.
[44] Zhang X, Takegoshi K, Hikichi K. Macromolecules 1991;24:5756.
[45] Zhang X, Takegoshi K, Hikichi K. Macromolecules 1993;26:2198.
[46] Zhang X, Solomon DH. Macromolecules 1994;27:4919.