pH Effect on the Mechanical Performance and Phase Mobility of Thermally Processed Wheat Gluten-Based Natural Polymer Materials

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

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

Received May 14, 2006; Revised Manuscript Received July 13, 2006

The mechanical properties, phase composition, and molecular motions of thermally processed wheat gluten- (WG-) based natural polymer materials were studied by mechanical testing, dynamic mechanical analysis (DMA), and solid-state NMR spectroscopy. The performance of the materials was mainly determined by the denaturization and cross-linking occurring in the thermal processing and the nature or amount of plasticizers used. The pH effect also played an important role in the materials when water was used as the only plasticizer (WG-w). Alkaline conditions modified the chemical structure of WG, possibly via deamidation; enhanced the thermal cross-linking of WG macromolecules to form a more stable aggregation structure; and promoted intermolecular interactions between water and all components in WG (proteins, starch, and lipid), resulting in a strong adhesion among different components and phases. The saponification of lipid under alkaline conditions also enhanced the hydrophilicity of lipid and the miscibility among lipid, water, and WG components. However, when glycerol was used with water as a plasticizer (WG-wg), the phase mobility and composition of the materials mainly depended on the content of glycerol when the water content was constant. During thermal processing under either acidic or alkaline conditions, glycerol was unlikely to thermally cross-link with WG as suggested previously. The advanced mechanical performance of the WG-wg materials was attributed to the nature of hydrogen-bonding interactions between glycerol and WG components in the materials. This caused the whole material to behave like a strengthened "cross-linked" structure at room temperature due to the low mobility of glycerol. The pH effect on phase mobility and compositions of WG-wg systems was not as significant as that for WG-w materials.

Introduction

Because of good viscoelastic properties, strong tensile strength, and excellent gas barrier properties, wheat gluten (WG) has been used as an edible film and has great potential to be applied as a renewable and biodegradable natural polymer material in food packaging, coating, and binding applications.[1−10] Plasticizers are always used in WG film casting to reduce the strong intra/intermolecular interactions among wheat protein molecules and to increase the mobility of the protein chains, thereby improving the flexibility and extensibility of the material. A comprehensive investigation of the formation of plasticized WG films has been reported regarding the use of different plasticizers, film formation solutions, thermal effect, pH effect, and conformation changes of WG during the film formation process.[11−25] Among them, the pH displayed a significant impact on the performance of WG films. It was indicated that the films formed under alkaline conditions exhibited stronger tensile strength than those formed under acidic conditions,[26,27] which was attributed to the protein solubility and/or the dispersion capability being enhanced under alkaline conditions.[26,28,29] The formation of dehydro- and cross-linked amino acids (such as lysinoalanine through condensation of lysine residues with dehydroalanine) could also happen during thermal heating under alkaline conditions to form intra- rather than intermolecular cross-linking in WG films.[30]

Thermal processing technologies such as compression molding or extrusion would produce WG-based materials more efficiently and avoid the use of a large amount of solvents. Although the methodologies have been used in food processing, only a few studies have been reported using the method to produce WG-based films or sheets.[15,16,31−35] Less effort has been paid to understanding the molecular motions, phase compositions, and mechanical performance related to pH effect, heating conditions, and shear forces used in processing. In this paper, plasticized WG materials with either water or water/glycerol as plasticizer were prepared by a compression molding method at pH = 4 or 11. The two pH conditions were selected because they are far away from the isoelectric point. Solid-state NMR, as a powerful technique for multicomponent/phase polymer systems,[34−42] was applied to explore the phase mobility and composition of the materials, the behavior of plasticizers, and the intermolecular interactions between plasticizers and WG components. The mechanical properties and the glass transition temperature (T_g) of the plasticized WG were also measured and correlated to the information on molecular motions and phase composition obtained from the NMR study. The possible chemical linkages formed between plasticizers and WG during thermal processing under different pH conditions were also examined. The pH effect on plasticization, phase mobility/composition, and mechanical performance of the materials was discussed.

Experimental Section

Materials. Wheat gluten vital (WG), supplied by Manildra Group Australia, contained about 80% proteins, 15% residual starch, 4% lipid, and around 1% fibers and other impurities on a dry basis. The moisture

* Corresponding author: tel +613 95452653; fax +613 95441128; e-mail Xiaoqing.Zhang@csiro.au.

10.1021/bm0604698 CCC: $33.50
Published on Web 11/09/2006

content was 12%. Sheet samples of the WG system were prepared with either water (WG-w) or water/glycerol (WG-wg) as plasticizers. The amount of water was varied in WG-w systems, while in WG-wg systems the water amount was fixed while the glycerol amount was varied. The pH of the plasticizers was adjusted to 4.0 with acetic acid or to 11 with NaOH. A small amount of Na_2SO_3 (0.3 wt % to WG) was added into all systems in order to dissociate the disulfide bonding within the protein chains to achieve efficient mixing between proteins and plasticizers.[43,44] Each sample with a designed formulation was mixed with a high-speed mixer operated at a speed of 3000 rpm for 1 min, left overnight, and then compression-molded at an optimum temperature of 130 °C for 5 min by use of a heating press with a pressure of 12 tons. The sample size was 145 mm × 145 mm with a thickness of 1.0 mm ± 0.1 mm. Moisture loss for WG-w systems could occur during thermal compression and testing at room temperature with RH of 50%; the water content for each sample was measured by drying the sample at 105 °C for 24 h after preparation of the sample via compression molding, and then after completion of all tests (in 2−4 days, stored in sealed plastic bags) as a final moisture content reported in the text. For WG-w samples minor further moisture loss occurred during testing, especially when water content was high, but the moisture content in WG-wg systems was relatively stable (10−12%). The plasticizer content for WG-wg systems was reported with the assumption that no glycerol loss occurred during the process.

Instrumentation. Tensile strength of the sheet samples was measured on an Instron 5566P at room temperature at ∼ 50% RH with a cross-head 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.

DMA experiments were operated on a Perkin-Elmer Pyris Diamond DMA in dual cantilever bending mode at a frequency of 1 Hz. The temperature range was set at −100 to 150 °C with a heating rate of 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.

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 a repetition of 2 s. The FID (free induction decay) signal of each sample was obtained by a solid-echo pulse sequence[45] and a Carr−Purcel−Meiboom−Gill [CPMG: 90°x-(t_1-180°y-t_1-echo)n] pulse sequence,[45] respectively, at 40 °C. The 90°−90° pulse spacing in the solid-echo was 10 μs and the dwell time was 1.25 μs. The 90°−180° pulse spacing (t_1) in the CPMG sequence was 50 μs, n was varied, and a total of 8 scans was used for each measurement. The whole FID of each sample was a combination of the data observed from solid-echo (time range of 0−0.15 ms) and from CPMG (time range of 0.4−8.0 ms) pulse sequences, and then a multidecay function was used to best fit the FID data with the IGOR program from Wave Metrics Inc.

High-resolution solid-state NMR experiments were conducted at room temperature on 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 by use of a single 90° pulse excitation (SPE) method with high-power decoupling. The 90° pulse was 4.5 μs for ^{1}H and ^{13}C (rf strength for spin-locking was 56 kHz), while the spinning rate of MAS was around 6−7 kHz. A contact time of 1.0 ms was used for measuring all CP/MAS spectra while the repetition time was 2 s and the dwell time was 2.5 μs. The chemical shift of ^{13}C spectra was determined by taking the carbonyl carbon of solid glycine (176.03 ppm) as an external reference standard. ^{1}H MAS NMR spectra were obtained with the same MAS rates and tetramethylsilane (TMS) was used as an external chemical shift reference. ^{1}H spin−spin (T_2) relaxation times under high-resolution conditions (MAS) were measured either through the decay of ^{13}C magnetization prepared by CP with varied CP delay times, as reported previously,[35,46] or through the decay of ^{1}H intensities in MAS spectra observed by the CPMG (t_1 of 40 μs) pulse sequence with a repetition time of 2 s and a 90° pulse length of 2.5 μs. ^{1}H solution NMR spectra were measured on the same spectrometer with a solution probe head. The T_2 values were obtained from fitting the intensities of resonances and no spectral deconvolution was applied.

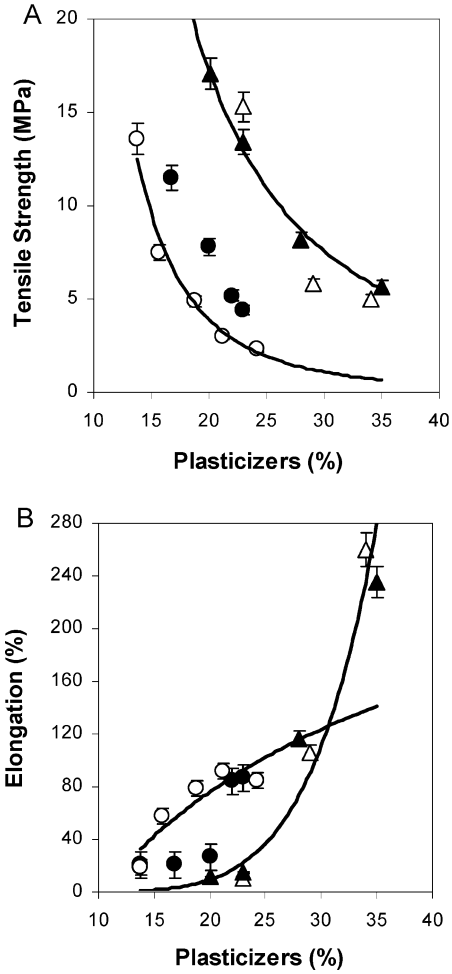

Figure 1. Tensile strength (A) and elongation at break (B) of the plasticized WG-w samples as a function of the plasticizer content at pH = 4 (○) or pH = 11 (●) and those of the WG-wg samples at pH = 4 (△) or pH = 11 (▲).

Results and Discussion

1. Mechanical Properties of Plasticized WG Materials. Plasticized wheat gluten materials present typical polymer properties, and their performance strongly depends on the nature or the amount of plasticizer in the system. The mechanical properties (tensile strength and elongation at break) of the WG-w and WG-wg materials are summarized in Figure 1. As predicted, the tensile strength of the materials decreased while the elongation of the materials increased as the amount of plasticizer increased. However, the WG-w systems always exhibited weaker tensile strength compared to WG-wg systems when a similar amount of plasticizer was used, being consistent with the more efficient plasticizing effect of water. The elongation of WG-w systems was longer than those of WG-wg systems at low plasticizer content, but it became shorter at high plasticizer content. The pH also played a significant role in the mechanical properties of WG-w materials; the tensile strength of materials at pH = 11 was always higher than that at pH = 4 in conjunction with shorter elongation at break, quite consistent with those reported for solution-cast WG films.[26−29] However, the me-

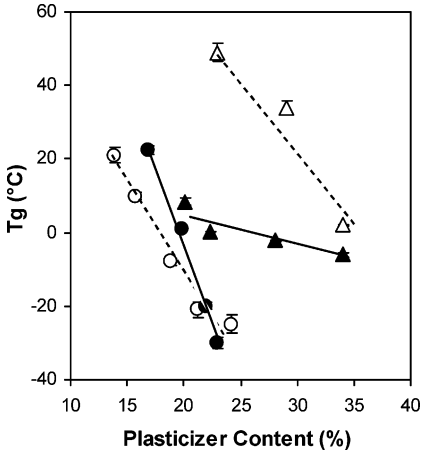

Figure 2. T_g values of plasticized WG-w samples as a function of the plasticizer content at pH = 4 (○) or pH = 11 (●) and those of WG-wg samples at pH = 4 (△) or pH = 11 (▲).

chanical performance of WG-wg materials mainly depended on the amount of plasticizers used and showed no pH dependence.

The T_g values of the WG systems are shown in Figure 2. The data were measured by DMA under the conditions reported previously,[34,35] where the onset of the storage modulus E' decrease, corresponding to T_g transition in DMA measurement, was taken as the T_g indicator. In all systems T_g decreased as the content of plasticizers increased, but the T_g value of WG-w samples decreased much faster than those of WG-wg, consistent with the more efficient plasticizing effect of water. The pH had a significant effect on the T_g obtained as evidenced by the WG-w samples with low water content displaying a lower T_g at pH = 4 than those at pH = 11. However, under alkaline condition (pH = 11) T_g decreased rapidly as the amount of plasticizers increased. When the water content exceeded 20 wt %, the T_g values at pH = 11 became similar to those at pH = 4. The T_g of WG-wg systems also displayed a pH dependence; the T_g values at pH = 11 were all lower and decreased slowly with increasing amounts of plasticizer, whereas at pH = 4 the T_g decreased rapidly with increasing plasticizer content. As the plasticizer content approached 35%, the values of T_g for the WG-wg systems became similar.

The general T_g trend obtained is consistent with that for solution-cast WG films, where alkaline conditions are favorable to plasticization and formation of strong materials. However, it is difficult to explain the similar mechanical behavior of the WG-wg materials thermally processed under both acidic and alkaline conditions. A detailed examination of phase mobility and composition is required for understanding the mechanical performance of these materials.

2. Phase Mobility and Composition. In a complex polymer system such as plasticized wheat gluten, various phases (such as plasticized phase, partially plasticized phase, and nonplasticized phase) would coexist and the molecular motions of these different components could be varied over a broad range due to their differences in chemical nature (proteins, starch, lipid, plasticizers), chain aggregation, and different intra- and intermolecular interactions within the components. Such materials normally show a broad T_g transition; thus, phase mobility and composition become very important for understanding and designing the properties of the materials. Solid-state NMR has provided a powerful tool to study the molecular motions and phase composition of these multicomponent/phase polymer materials.

^{1}H spin−spin relaxation (T_2) is sensitive to the mobility of polymers at molecular levels.[45] The ^{1}H T_2 value of a polymer

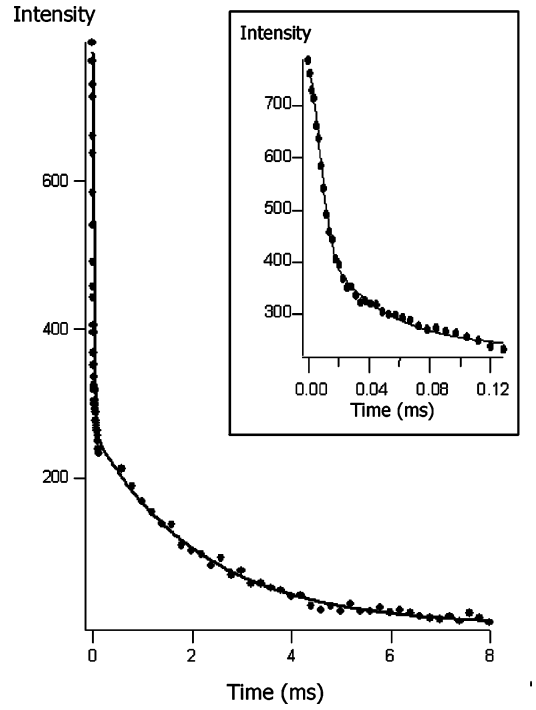

Figure 3. Best fitting result and experimental FID data of a plasticized WG-w sample containing 20% water at pH = 11 as observed via a combination of signals observed by solid-echo (with details shown in the inset) and CPMG pulse sequences.

usually ranges from 10 μs for rigid polymers (below T_g), increases significantly at T_g transitions, and reaches above 1−100 ms for rubbery or viscoelastic components. A complex system with various components in a wide range of mobility would show multicomponent free induction decays (FID). The analysis of the FID signal would provide information of both motional nature (T_2 value) and proportion (the intensity of the T_2 component) of each component.[45] In order to detect all components in the WG systems, their FID signals were measured by a combination of those detected via solid-echo (sensitive to rigid components) and CPMG (sensitive to mobile components). The complete FID data in conjunction with fitting results of WG-w(20%) at pH = 11 observed by this method are shown in Figure 3 as a typical example. Three components were indeed obtained by best fitting, including a Gaussian decay with a short T_2 (T_{2S}) and two exponential decays with longer T_2 values (T_{2M} and T_{2L}), which could be correlated to rigid, intermediate, and mobile phases. The error was mainly due to the signal-to-noise ratio (s/n) of the spectra, and it was around 3−5% when the number of scans was 8 in the measurement.

The ^{1}H T_2 data (including T_{2S}, T_{2M}, and T_{2L} values and the proportion of each component as phase composition) of WG-w systems at pH = 4 or 11 are shown in Figure 4. In WG-w systems, all three T_2 components behaved differently with strong pH dependence. The T_{2S} proportion decreased as the amount of water increased under both pH conditions; however, when water content was low, the system at pH = 11 had a larger proportion of T_{2S} phase, although it decreased faster as the water content increased. When the water content was around 23−25 wt %, there was about 20−25 ^{1}H % T_{2S} component in the samples at both pH conditions where the T_{2S} value increased only slightly (from 9.5 to 13 μs). The T_2 values of the T_{2M} component remained in the range of 150−160 μs for WG-w samples at pH = 4, but the values were around 30−40 μs at pH = 11 suggesting the T_{2M} phase was not as mobile as that at pH = 4. The pH effect was also present for T_{2L}; the T_{2L} values

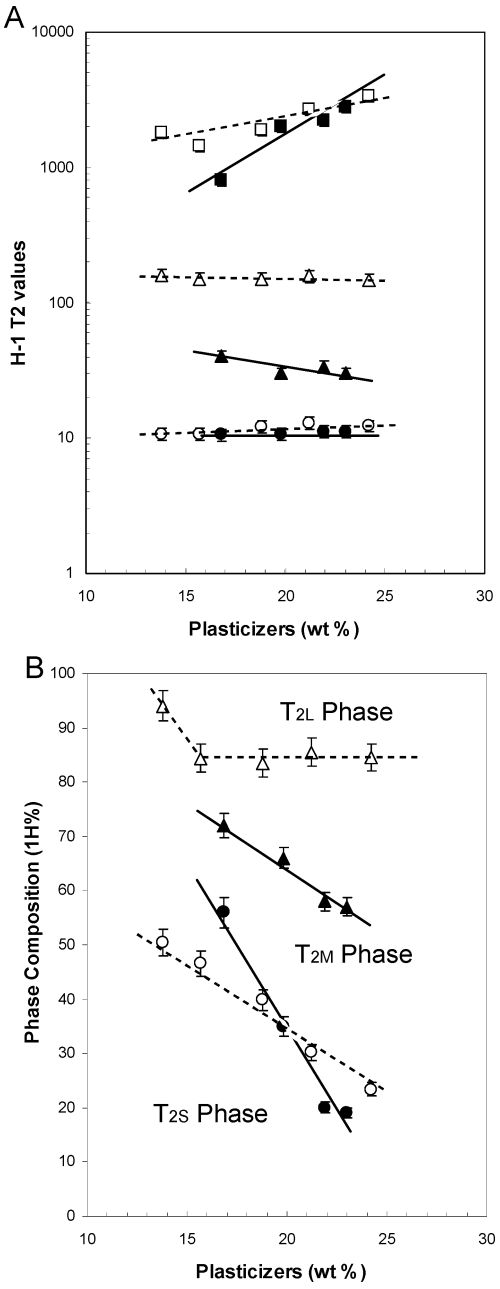

Figure 4. (A) ^{1}H T_{2S}, T_{2M}, and T_{2L} values (microseconds) of WG-w systems at pH = 4 (○, △, □) or pH = 11 (●, ▲, ■). (B) Proportions of T_{2S} or (T_{2S} + T_{2M}) phases of WG-w systems at pH = 4 (○ or △) or pH = 11 (● or ▲), where total proportions of (T_{2S} + T_{2M} + T_{2L}) = 100%.

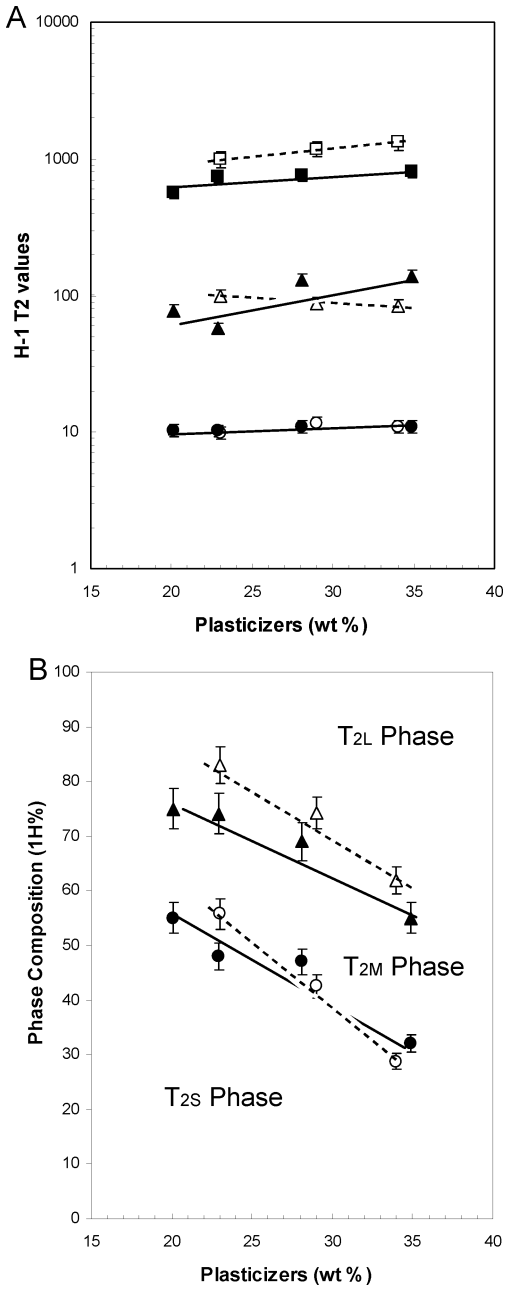

Figure 5. (A) ^{1}H T_{2S}, T_{2M}, and T_{2L} values (microseconds) of WG-wg systems at pH = 4 (○, △, □) or pH = 11 (●, ▲, ■). (B) Proportions of T_{2S} or (T_{2S} + T_{2M}) phases of WG-wg systems at pH = 4 (○ or △) or pH = 11 (● or ▲), where total proportions of (T_{2S} + T_{2M} + T_{2L}) = 100%.

were always higher at pH = 4 until the water content reached 25 wt %. The proportion of T_{2L} at pH = 4 decreased initially when the water content increased, and then it remained constant around 15−16 ^{1}H %. The proportion at pH = 11 increased significantly when the water content increased and reached 47 ^{1}H % at a water content of 23 wt %. It seems that the more stable aggregated WG structure formed under alkaline conditions was more difficult to plasticize when the amount of plasticizer was low.

Phase mobility and composition for WG-wg systems as described in ^{1}H T_2 data at pH = 4 and pH = 11 are shown in Figure 5B. The proportion of T_{2S} phase (the rigid phase) was always higher in WG-wg than in WG-w systems. It is interesting to note that the results mainly relied on the content of plasticizers (glycerol, as the water content was constant). The pH showed only a minor effect on the performance: T_{2L} values were slightly longer while the proportions were also lower at pH = 4. The rigid and intermediate phases (T_{2S} and T_{2M}) of the systems at the two pH conditions showed similar behavior. Increasing the plasticizer content under both pH conditions decreased the proportion of rigid phase and increased the proportion of the mobile phase, but the proportion of the intermediate phase remained almost constant.

The T_g values of most of the WG samples were below 40 °C, the temperature at which T_2 was measured, and the T_g values of the systems with high plasticizer content were even below 0 °C. However, rigid, intermediate, and mobile components all coexisted at 40 °C, indicating some components of WG still remained unplasticized and did not undergo T_g transition at temperatures far above the average T_g of the materials. The different plasticizing effects in these components caused a wide distribution of mobility in the various phases in the WG

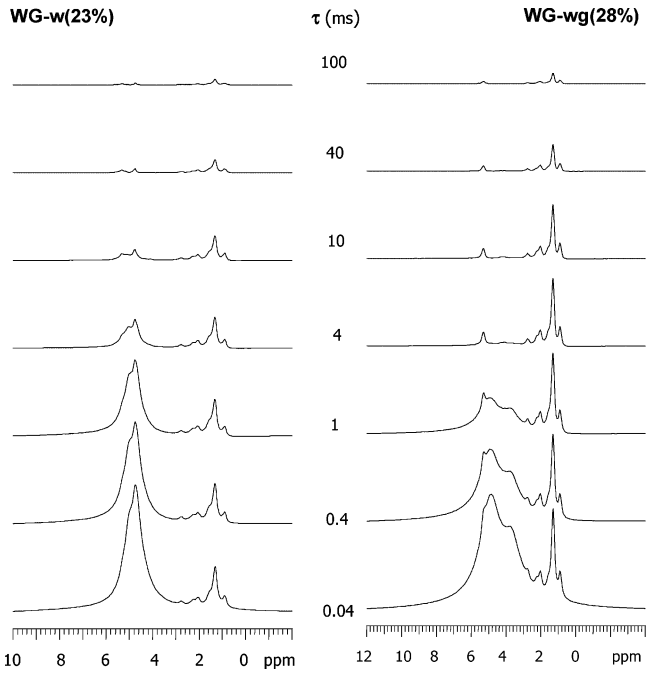

Figure 6. ^{1}H MAS NMR spectra of WG-w(23%) and WG-wg(28%) sample at pH = 11, observed by CPMG pulse sequence under varied τ delay times.

materials, which contributed to the broad tan δ peak corresponding to the T_g transition in the DMA measurement. Usually DSC and DMA provide motional information of polymer components over a scale of 20−100 nm, while ^{1}H T_2 is very sensitive to local dipolar interactions at the molecular level (a scale below 1 nm). The two methodologies provide dynamic information of a material on different scales. The result indicates that phase mobility and compositions of the plasticized WG materials had a direct relationship with the mechanical performance. The stronger tensile strength of WG-w at pH = 11 could be due to the lower mobility of T_{2M} and T_{2L} phases in the materials, while the similar mechanical performances of the WG-wg systems were attributed to their similar phase mobility and composition at both pH conditions.

Most of the previous ^{1}H T_2 or FID data on WG systems were obtained by either a single 90° pulse[37,39,41] (where the dead time of a pulse could be a problem to detect the rigid components with T_2 around 10 μs, in conjunction with the possibility of missing the very mobile components), or a solid-echo (or so-called quadrupolar echo),[42] which overcame the problem of dead time but still experienced difficulties in measuring very mobile components. The method to combine signals observed from both solid-echo and CPMG to obtain the FID data used in this work should be a suitable way to detect all polymer components in a complex system with a wide distribution of mobility.

3. Motional Behavior of Plasticizers. To examine the behavior of plasticizers in WG systems is another important element in understanding the plasticizing effects on the WG materials. High-resolution solid-state NMR technique provides an advanced method to explore the behavior of each individual component in a complicated system with good resolution and selectivity. In the WG systems studied here, water and glycerol were used as plasticizers, and the lipid in WG may also play a role of plasticizer as suggested in previous reports.[15,19] These small molecules could contribute to the mobile phase in the materials, which should be detected in ^{1}H MAS NMR spectra. Figure 6 shows the ^{1}H MAS spectra of WG-w(23%) and WG-wg(28%) at pH = 11 measured by CPMG pulse sequence with several typical τ times at which the nth echo appeared. The rigid phase (proteins or starch) with a short T_2 value (broad line width) would be missed in the spectra. The strong peak at 4.5 ppm was assigned to water for WG-w systems, while the broad peaks at 4.5−3.7 ppm are due to water and glycerol (WG-wg) with a fast chemical exchange between water and glycerol. The resonances at 0.9, 1.3, 2.0, 2.7, and 5.3 ppm were all assigned to lipid.[26] As the τ time increased, the intensity of the water or water/glycerol peak decayed much faster than those of lipid resonances, indicating lipid was more mobile than water and glycerol in the systems. Examination of the ^{1}H T_2 behavior of these components can provide their motional information and interactions with WG components.

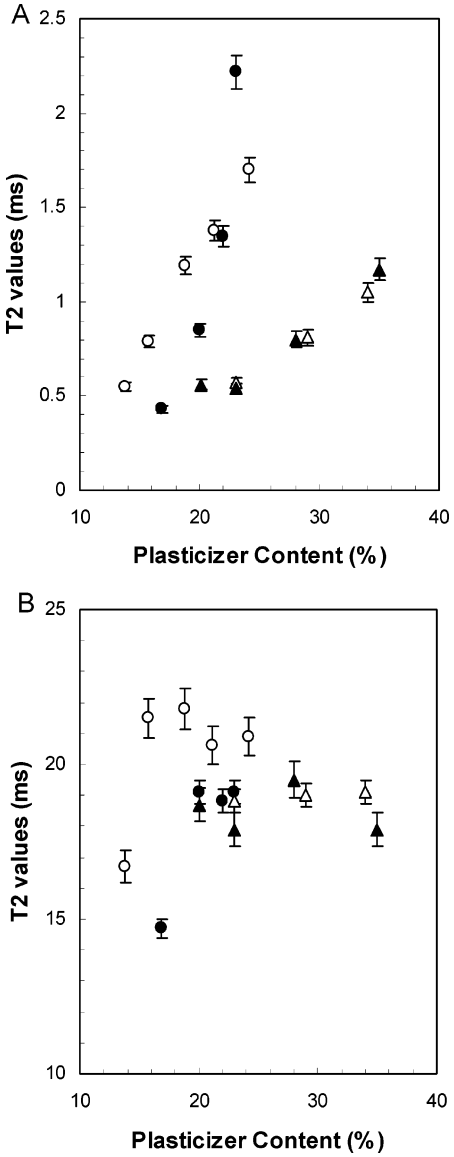

Figure 7. ^{1}H T_2 of the mobile components observed through ^{1}H MAS spectra. (A) T_2 of water or water/glycerol at pH = 4 or pH = 11 for WG-w (○, ●) or WG-wg systems (△, ▲). (B) T_2 of lipid (at 1.3 ppm) at pH = 4 or pH = 11 for WG-w (○, ●) or WG-wg systems (△, ▲).

The ^{1}H T_2 data of water or water/glycerol (at 4.5−3.7 ppm) and lipid (at 1.3 ppm) for the WG systems are shown in Figure 7. In general, the T_2 of water or water/glycerol increased with increasing plasticizer content, but the values were always much lower than those of lipid in all systems. For WG-w systems, the T_2 of water increased faster at pH = 11 than at pH = 4 as the water content increased. At the same time, the values of lipid at pH = 4 were longer than those at pH = 11. The longer

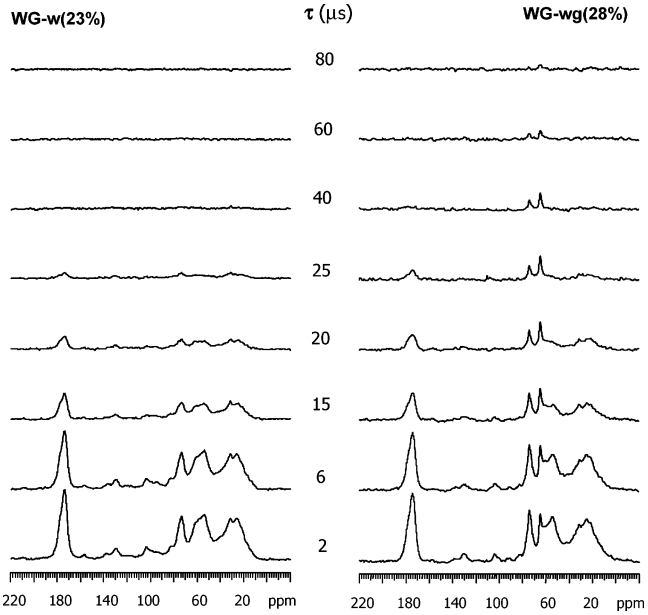

Figure 8. ^{13}C CP/MAS NMR spectra of WG-w(23%) and WG-wg-(28%) at pH = 11, measured with varied CP delay times (τ) where the intensity decay follows ^{1}H T_2 decay. (CP contact time 1 ms, spinning rate 6.6 kHz).

T_{2L} values for the WG-w system at pH = 4 (shown in Figure 4) indicated the high mobility of lipid and weaker interactions with other components in the materials. The behaviors of both the lipid and the plasticizers in WG-wg systems were quite similar at both pH conditions, being consistent with their similar phase mobility and compositions. Note that the T_2 values of water or water/glycerol measured under MAS conditions were similar to those detected by broad-line NMR under static conditions. This is consistent with the line width broadening mechanism of the water and glycerol plasticizers resulting from chemical exchange between sites with different chemical shifts or relaxation processes arising from motional heterogeneous regimes and various interaction species in the systems.[26,30,31]

In a recent paper on the WG-wg system at pH = 4,[35] besides observing the mobile phase in SPE spectra, glycerol signals were also detected by ^{13}C CP/MAS NMR spectra, which are sensitive to rigid phases with strong dipolar interactions that are capable of transferring their proton magnetization to ^{13}C resonances. It suggested strong hydrogen bonding between WG and glycerol, and possibly some glycerol molecules even existed in the WG rigid phase. By use of the same method as in ref 35, the ^{1}H T_2 values of WG-w and WG-wg systems at pH = 11 were measured via ^{13}C CP/MAS NMR with varied CP delay times (in total, 16 values between 2 and 100 μs). Some spectra of WG-w(23%) and WG-wg(28%) are shown as examples in Figure 8. With short CP delay times (ca. 2 μs), mainly signals of proteins and starch were detected for the WG-w system. These signals decayed very rapidly as the CP delay time increased following a single Gaussian decay model with ^{1}H T_2 values around 13−15 μs, corresponding to the T_{2S} phase in Figure 4. The signals of glycerol at 74 and 64 ppm (narrow lines) were indeed observed for WG-wg systems at short CP delay times. In contrast to the resonances of proteins and starch, which followed a single Gaussian decay with increasing CP delay time, the narrow glycerol resonance at 64 ppm displayed two T_2 components following a model of one Gaussian plus one exponential decay function with similar ^{1}H T_2 values as reported for WG-wg at pH = 4.[35] The results indicated that, under both acidic and alkaline conditions, the plasticizer could exist in all phases (rigid, intermediate, and mobile) to different extents, and strong adhesion among different phases could be formed through hydrogen-bonding interactions.

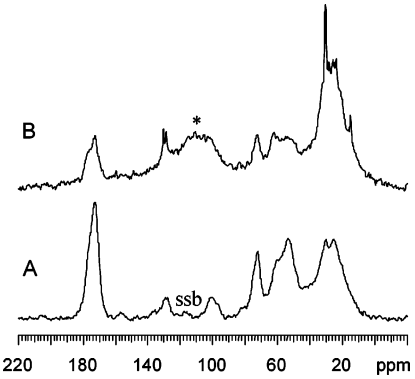

Figure 9. ^{13}C CP/MAS (A) and SPE (B) NMR spectra of WG-wg at pH = 11 after removal of plasticizers. (ssb, spin-side band; *, background signal of the spinner; spinning rate, 6.6 kHz; CP contact time, 1 ms; SPE relaxation delay, 2 s).

4. pH Effect on the Structure of Wheat Gluten. It was suggested that glycerol could take part in the thermal cross-linking reactions of WG components under heating conditions that stabilized the systems and contributed to the strength of the materials.[30,31] This seemed consistent with the results that WG-wg systems always displayed strong tensile strength and higher T_g than those of WG-w materials. In order to provide detailed information of the structures of WG materials formed under these different pH conditions with no plasticizing effect, the samples were also examined by solid-state NMR techniques after plasticizers were removed from the WG systems as described below.

Samples WG-w(22%) at pH = 4, WG-w(23%) at pH = 11, WG-wg(29%) at pH = 4, and WG-wg(28%) at pH = 11 were soaked in distilled water (5% solid) under sonication. These samples were then washed by distilled water and dried at room temperature to a constant moisture content around 4%. Further drying of the samples was avoided as it might add another factor to change the WG structures. These samples were labeled as WG-w pH = 4 and pH = 11 and WG-wg pH = 4 and pH = 11. In addition, WG sheet samples compression-molded under the same conditions with 20% water or 20% glycerol + 10% water as plasticizer were also treated according to the same procedure and labeled as WG-w pH = 6 (pH of WG is around 5−6) or WG-wg pH = 6. The glycerol content in the water after sonication of WG-wg samples was measured by ^{1}H solution NMR spectroscopy. In all cases, the amount of glycerol in water after sonication for 1 h was quite close (within 1−2%) to the amount added to the systems during thermal processing. This indicated that even if glycerol might take part in the thermal cross-linking reactions with WG, the amount would be very small. Most of the glycerol molecules were hydrogen-bonded to the WG without chemical bonding. Additionally, only a very small amount (less than 2%) of WG (mainly starch) was obtained in the water phase after sonication for 5 h, in contrast to 35% of WG dissolved under the same conditions for WG powder without thermal processing. This indicates significant thermal cross-linking for WG did occur during the compression molding to form a stable denatured cross-linked network through the materials.

All six samples obtained by the above procedure displayed similar CP/MAS and SPE ^{13}C NMR spectra; those of WG-wg pH = 11 are shown in Figure 9 as a typical example. In both spectra, all glycerol signals disappeared. In the CP/MAS

Table 1. ^{1}H T_2 Values[a] Observed via ^{1}H MAS Spectra for WG Systems after Removal of Plasticizers

samples	5.3 ppm	2.7 ppm	2.2 ppm	2.0 ppm	1.3 ppm	0.9 ppm
WG-w, pH = 4	24.2	12.6	11.3	21.7	30.0	26.9
WG-w, pH = 6	14.4	7.0	8.8	16.2	27.5	21.5
WG-w, pH = 11	11.1	5.6	6.5	12.2	20.7	16.9
WG-wg, pH = 4	21.1	9.0	8.5	16.5	24.6	21.1
WG-wg, pH = 6	14.4	7.0	6.6	16.2	24.8	21.5
WG-wg, pH = 11	12.3	6.1	6.1	14.2	22.1	19.9

[a] T_2 values are given in milliseconds.

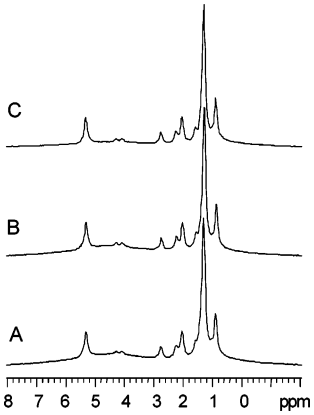

Figure 10. ^{1}H MAS NMR spectra of WG-w at (A) pH = 4, (B) pH = 6, and (C) pH = 11 after removal of plasticizers.

spectrum, resonances at 173, 54, and 30−25 ppm are attributed to the proteins while those at 103, 83, and 74 ppm are due to residual starch. Mainly mobile lipid resonances were detected in the SPE spectrum (at 179, 130, 30−23, and 15 ppm) in conjunction with a minor amount of mobile proteins (174, 60−45, and 30−15 ppm) and starch (75 ppm). The intensities at 61 and 52 ppm in the SPE spectrum indicated the presence of proline and glutamine in the mobile state, being consistent with previous reports that proline and glutamine were the easiest segments in wheat proteins to plasticize or hydrate.[24,30,31]

The pH effect on the lipid component after removal of plasticizers was also examined. Figure 10 shows the ^{1}H MAS spectra of WG-w pH = 4, 6, and 11, where only lipid signals were obtained while the rigid components appeared as a very broad baseline. The spectra for WG-w systems displayed similar behavior. The ^{1}H T_2 values of the lipid in these samples were obtained by CPMG pulse sequence via ^{1}H MAS spectra and are listed in Table 1. In both WG-w and WG-wg systems the ^{1}H T_2 decreased as pH increased for most lipid resonances. The ^{1}H T_2 data were also quite similar under the same pH conditions no matter whether in the WG-w or the WG-wg system. Alkaline conditions would cause saponification of lipid and increase the hydrophilicity of the component. This would promote its interactions with WG and plasticizers; thus the mobility of the lipid molecules would be reduced to some extent. Note that the ^{1}H T_2 values of the lipid became longer after removal of plasticizers, suggesting the lipid did interact with plasticizers and became more mobile when the plasticizers were removed from the systems.

The amount of water would play an important role in the pH effect on the performance of the WG materials. Deamidation under alkaline conditions would convert glutamine amide groups (i.e., protein−$CONH_2$) to glutamate carboxylate groups (i.e., protein−COO^-) that reduced the hydrogen bonding within protein structures, thus enhancing the solubility and dispersion capability of WG during solution casting, which would favor formation of stronger films.[26−29] In WG thermal processing, thermal cross-linking was predominant. When a sufficient amount of water was present (high water content in WG-w samples), most of the processes under alkaline conditions could also occur to some extent, resulting in additional strong aggregation within the WG chains via strong intermolecular interactions among these different components/phases in the materials. Thermal cross-linking should generate greater mechanical strength for such materials. However, in WG-wg systems the amount of glycerol was predominantly present as plasticizer, and the amount of water was low and constant. The alkaline conditions resulted in an efficient plasticization of a certain proportion of WG as shown in the T_g behavior (Figure 2), but the mechanical performance and phase mobility of the major proportion mainly depended on the amount of glycerol used. pH did not play a significant role for this major proportion. The chemical linkage via a cross-linking reaction suggested previously between glycerol and WG was not observed in the WG-wg systems. Hydrogen bonding between glycerol and WG macromolecules formed during thermal processing would be stronger than that between water and WG due to the lower mobility of glycerol at room temperatures. The whole materials behaved as though they had an additional cross-linking effect through the interactions, and the molecular motions of the plasticized (mobile) phases in WG-wg materials were restricted to a certain extent, thus resulting in greater mechanical strength.

Conclusions

The mechanical properties and phase structures of thermally processed WG materials were mainly determined by the significant denaturization and cross-linking that occurred during the thermal processing and by the nature or amount of plasticizer used. The pH effect played an important role in WG-w materials. As in solution casting, alkaline conditions caused some level of deamidation of WG that modified the chemical and aggregation structures, enhanced intermolecular interactions between water and all components in WG (proteins, starch, and lipid), and resulted in a more stable cross-linked WG network with strong intermolecular interactions (adhesion) among the different components and phases. Thus, the materials displayed greater mechanical strength. The saponification that occurred under alkaline conditions also enhanced the hydrophilicity of lipid and the miscibility among lipid, water, and WG components, thus also playing a role in the results obtained. However, for WG-wg materials with a low constant amount of water, the performance of materials was not significantly affected by pH. The phase mobility/composition mainly depended on the content of glycerol. During thermal processing under either acidic or alkaline conditions, glycerol was unlikely to thermally cross-link with WG under the conditions used in this work. The advanced mechanical performance of WG-wg materials was attributed to the strong hydrogen-bonding interactions between glycerol and WG components present in all phases acting as an additional cross-linking effect on the whole material.

References and Notes

(1) Belton, P. *J. Cereal Sci.* **1999**, *29*, 103−107.
(2) Ornebro, J.; Nylander, T.; Eliasson, A. *J. Cereal Sci.* **2000**, *31*, 195−221.
(3) Singh, H.; MacRitchie, F. *J. Cereal Sci.* **2001**, *33*, 231−243.
(4) Scott, G. *Degradable Polymers: Principles and Applications*, 2nd ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2002.
(5) Kersting, H.; Lindhauer, M. G.; Bergthaller, W. *Ind. Crops Prod.* **1994**, *3*, 121−128.
(6) Derksen, J. T. P.; Cuperus, F. P.; Kolster, P. *Ind. Crops Prod.* **1995**, *3*, 225−236.
(7) Derksen, J. T. P.; Cuperus, F. P.; Kolster, P. *Prog. Org. Coat.* **1996**, *27*, 45−53.
(8) Gennadios, A; Weller, C. L.; Testin, R. F. *Cereal Chem.* **1993**, *4*, 426−429.
(9) Park, H. J.; Chinnan, M. S. *J. Food Eng.* **1995**, *25*, 497−507.
(10) Roy, S.; Gennadios, A.; Weller, C. L.; Testin, R. F. *Ind. Crops Prod.* **2000**, *11*, 43−50.
(11) Ali, Y.; Ghorpade, V. M.; Hanna, M. A. *Ind. Crops Prod.* **1997**, *6*, 177−184.
(12) Gontard, N.; Ring, S. *J. Agric. Food Chem.* **1996**, *44*, 3474−3478.
(13) Pouplin, M.; Redl, A.; Gontard, N. *J. Agric. Food Chem.* **1999**, *47*, 538−543.
(14) Irissin-Mangata, J.; Bauduin, G.; Boutevin, B.; Gontard, N. *Eur. Polym. J.* **2001**, *37*, 1533−1541.
(15) Pommet. M.; Redl, A.; Morel, M. H.; Guilbert, S. *Polymer* **2003**, *44*, 115−122.
(16) Redl, A.; Morel, M.; Bonicel, J.; Vergnes, B.; Guibert, S. *Cereal Chem.* **1999**, *76*, 361−370.
(17) Redl, A.; Morel, M.; Bonicel, J.; Guibert, S.; Vergnes, B. *Rheol. Acta* **1999**, *38*, 311−320.
(18) Zhang, X.; Burgar, I.; Do, M.; Lourbakos, E.; Beh, H. *Polym. Prepr.* **2003**, *44* (1), 402−403.
(19) Pommet, M.; Redl, A.; Guibert, S.; Morel, M.-M. *J. Cereal Sci.* **2005**, *42*, 81−91.
(20) Apichartsrangkoon, A.; Ledward, D.; Bell, A.; Brennan, J. *Food Chem.* **1998**, *63*, 215−220.
(21) Graaf, L. *J. Biotechnol.* **2000**, *79*, 299−306.
(22) Elizalde, B. E.; Pilosof, A. M. R. *J. Food Eng.* **1999**, *42*, 97−102.
(23) Hargreaves, J.; Popineau, Y.; Meste, M.; Hemminga, M. *FEBS Lett.* **1995**, *372*, 103−107.
(24) Micard, V.; Guilbert, S. *Int. J. Biol. Macromol.* **2000**, *27*, 229−236.
(25) Lens, J.-P.; de Graaf, L. A.; Stevels, W. M.; Dietz, C. H. J. T.; Verhelst, K. C. S.; Vereijken, J. M.; Kolster, P. *Ind. Crops Prod.* **2003**, *17*, 119−130.
(26) Gennadios, A; Brandenburg, A. H.; Weller, C. L.; Testin, R. F. *J. Agric. Food Chem.* **1993**, *41*, 1835−1839.
(27) Kayserilioglu, B. S.; Stevels, W. M.; Mulder, W. J.; Akkas, N. *Starch* **2001**, *53*, 381−386.
(28) Herald, T. J.; Gnanasambandam, R.; McGuire, B. H.; Hachmeister, K. A. *J. Food Sci.* **1995**, *60*, 1147−1151.
(29) Roy, S.; Gennadios, A.; Weller, C. L.; Zeece, M. G.; Testin, R. F. *J. Food Sci.* **1999**, *64*, 57−60.
(30) Friedman, M. *J. Agric. Food Chem.* **1999**, *47*, 1295−1317.
(31) Micard, V.; Morel, M.-H.; Bonicel, J.; Guilbert, S. *Polymer* **2001**, *42*, 477−485.
(32) Gallstedt, M.; Mattozzi, A.; Johansson, E.; Hedenqvist, M. S. *Biomacromolecules* **2004**, *5*, 2020−2028.
(33) Zhang, X.; Do, M.; Lourbakos, E. *Polym. Prepr.* **2005**, *46* (1), 321−322.
(34) Zhang, X.; Burgar, I.; Lourbakos, E.; Beh, H. *Polymer* **2004**, *45*, 3305−3312.
(35) Zhang, X.; Burgar, I.; Do, M.; Lourbakos, E. *Biomacromolecules* **2005**, *6*, 1661−1671.
(36) Belton, P. S.; Duce, S.; Colquhoun, I. J.; Tatham, A. S. *Magn. Reson. Chem.* **1988**, *26*, 245−251.
(37) Abelett, S.; Barnes, D. J.; Davies, A. P.; Ingman, S. J.; Patient, D. W. *J. Cereal Sci.* **1988**, *7*, 11−20.
(38) Belton, P. S.; Duce, S.; Tatham, A. S. *J. Cereal Sci.* **1988**, *7*, 113−122.
(39) Belton, P. S.; Colquhoun, I. J.; Grant, A.; Weliner, N.; Field, J. M.; Shewry, P. R.; Tatham, A. S. *Int. J. Biol. Macromol.* **1995**, *17*, 74−80.
(40) Umbach, S. L.; Davis, E. A.; Gordon, J. *J. Cereal Sci.* **1998**, *28*, 233−242.
(41) Calucci, L.; Forte, C.; Galleschi, L.; Geppi. M.; Ghiringhelli, S. *Int. J. Biol. Macromol.* **2003**, *32*, 179−189.
(42) Calucci, L.; Galleschi, L.; Geppi. M.; Molloca, G. *Biomacromolecules* **2004**, *5*, 1536−1544.
(43) Gontard, N.; Guilbert, S.; Cuq, J. L. *J. Food Sci.* **1992**, *57*, 190−199.
(44) Micard, V.; Morel, M.-H.; Bonicel, J.; Guilbert, S. *Polymer* **2001**, *42*, 477−485.
(45) McBrierty, V.; Packer, K. *Nuclear Magnetic Resonance in Solid Polymers*; Cambridge University Press: Cambridge, U.K., 1993.
(46) Alexlson, D. E.; Russell, K. *Prog. Polym. Sci.* **1985**, *11*, 221−282.

BM0604698