

Effect of Glass Transition and Cross-Linking on Rheological Properties of Gluten: Development of a Preliminary State Diagram

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ABSTRACT

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The glass transition temperature of gluten at different moisture levels was determined by differential scanning calorimetry and mechanical spectrometry. The dynamic moduli (G' and G'') of gluten with 10–40% moisture were measured as a function of temperature by pressure rheometry. At 10% moisture, gluten exhibited entangled polymer flow at 92–140°C and networking reactions at higher temperatures. At higher moisture levels,

gluten experienced structured flow before networking cross-linking reactions. The onset temperature of the reaction zone was 120°C in 20% moisture gluten and 93°C at moisture levels of 30–40%. Softening of the vitrified network occurred at 184, 181, and 170°C in 20, 30, and 40% moisture gluten, respectively. A preliminary state diagram of gluten as a function of moisture and temperature was developed.

The unique viscoelastic properties of wheat flour doughs are governed by the gluten proteins. The classification of wheat flours as strong and weak and their suitability for different applications are largely determined by the rheological behavior of gluten (Bloksma and Bushuk 1988; Khatkar and Schofield 1997). Furthermore, the structure and texture of bread, cakes, crackers, and extruded wheat products are shaped, to a large extent, by the heat-induced changes in the gluten fraction of wheat flour (Davies 1986; Hosene 1989; Levine and Slade 1990; Li and Lee 1996; Nikolaidis and Labuza 1996). In addition to its universally recognized functionality in baked products, wheat gluten has been utilized in the development of biodegradable packaging materials because of its excellent film-forming properties (Cuq et al 1998).

Gluten comprises two major protein fractions that differ in solubility in concentrated aliphatic alcohol solutions. The gliadin fraction refers to proteins that are extractable by 70% ethanol, while glutenin comprises proteins that remain insoluble under these conditions (Schofield 1994). The gliadins are monomeric proteins with intrachain disulfide bonds, where present, while glutenins comprise polymeric proteins with component subunits linked by disulfide bonds. When hydrated, gliadin behaves as a viscous liquid while glutenin forms a cohesive elastic solid (Khatkar and Schofield 1997).

Like synthetic polymers, cereal and legume proteins exist in an amorphous metastable state sensitive to changes in moisture and temperature (Levine and Slade 1990; Cocero and Kokini 1991; Cherian and Chinachoti 1996; Madeka and Kokini 1996; Morales-Diaz and Kokini 1998). According to Sperling (1992), synthetic amorphous polymers exhibit five regions of temperature-dependent viscoelastic behavior. At low temperatures, the polymer is hard and brittle, or glassy, and becomes leathery and changes to a rubber at higher temperatures. The temperature at which the material changes from glass to leather is designated as the glass transition temperature (T_g). With a further increase in temperature, linear polymers traverse the rubbery flow region and ultimately become free-flowing (liquid flow region), while cross-linked polymers remain in the rubbery state up to the decomposition temperature of the polymer, especially as the network formed suppresses flow. Low molecular weight compounds compatible with the polymers plasticize the chains and lower the glass transition temperature. The most common plasticizer for hydrophilic polymers is water (Slade and Levine 1995).

Gluten and its subfractions, gliadin and glutenin, exhibit a glass transition (Hosene et al 1986; Cocero and Kokini 1991; de Graaf et al 1993; Elizalde and Pilosof 1999). At temperatures higher than T_g , gluten polymers acquire enough mobility and cross-link to form a network stabilized by disulfide bonds (Slade et al 1989; Levine and Slade 1990). Rheological and extractability studies on gluten, gliadin, and glutenin showed that heating promoted network formation through association of the polymer chains by hydrophobic interactions and disulfide bonds (Schofield et al 1983; Attenburrow et al 1990; Weegels et al 1994; Tsiami et al 1997a,b).

Mapping the changes in rheological properties of food biopolymers resulting from plasticization by water and other processing parameters and their presentation in state diagrams are pivotal for process design and evaluation, quality control, and definition of optimum storage conditions (Levine and Slade 1990). The state diagrams of gluten subfractions (gliadin and glutenin) were constructed (Kokini et al 1994, 1995). However, in systems using gluten or wheat flour as an ingredient, it is the interaction between gliadin and glutenin, along with the changes brought about in the resulting gluten network during processing, that defines the structure and texture of the final product. Consequently, the objective of the present work was the construction of a state diagram representing the order-disorder transitions and networking reactions of wheat gluten as a function of moisture and temperature.

MATERIALS AND METHODS

Sample Preparation

Wheat gluten (Lot 127H0169, Sigma Chemical Co., St. Louis, MO) with protein content of 80%, dry mass basis, was brought to moisture content of 50% and mixed in a Resistograph (Brabender Instruments, South Hackensack, NJ) at 63 rpm and 30°C for 10 min. The resulting gluten dough was fed into a single-screw extruder (Brabender) and extruded at 50 rpm and 50°C to shape it into strips that were subsequently left at room temperature to attain a moisture content of 10–12%. The extrusion parameters induced enough plasticity to move the material in the extruder. Mixing in a farinograph or a pin mixer, heating at 60°C and pressing at 3.1×10^8 Pa, and extrusion have been used in the preparation of gluten and glutenin samples for mechanical spectrometry (Cocero and Kokini 1991; Kalichevsky et al 1992; Cherian and Chinachoti 1996; Pouplin et al 1999). A portion of the extrudate was freeze-dried (model 50-SRC, Virtis, Gardiner, NY) to a moisture content of 2–3% and milled (model ZM1, Glenmills, Maywood, NJ) fitted with a 0.12-mm screen. The gluten strips and powders were equilibrated over P_2O_5 or saturated solutions of LiCl, CH_3COOK , K_2CO_3 , NaBr, NaCl, KCl, $BaCl_2$ or K_2SO_4 , which produced equilibrium relative humidities (ERH) of 0, 12, 23, 44, 52, 75, 84, 90, and 97%, respectively. Samples kept at $ERH \leq 84\%$ or $\geq 90\%$ were equilibrated at 25°C for 21 days or at 5°C for seven days, respectively.

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Disks were cut from gluten sheets prepared by pressing gluten doughs between Plexiglas plates to a thickness of 2-mm and storing at 5°C for 48 hr. The disks were equilibrated at room temperature or over saturated salt solutions to a final moisture content of 10, 20, 30, or 40%.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was conducted on a TA 4000 thermal analysis system with a DSC 30-S cell and a TC11TA processor (Mettler Instruments, Hightstown, NJ). The system, fitted with a liquid nitrogen controlled cooling accessory, was calibrated for temperature with n-hexane (mp -93.5°C), water (mp 0.0°C) and indium (mp 156.6°C), and for heat flow with indium (heat of fusion 28.45 J/g). Samples were placed in 40- μ L aluminum pans and scanned from -80 to 180°C at 5°C/min using an empty aluminum pan as a reference. Thermograms were analyzed by software (TA 72, Graphware, Cincinnati, OH). The glass transition was determined as the midpoint between onset and end temperatures of the endothermic shift in heat capacity.

Mechanical Spectrometry

A mechanical spectrometer (RMS-800, Rheometrics, Piscataway, NJ) fitted with a liquid nitrogen-controlled cooling accessory was used for analysis. Gluten strips (43.7 \pm 2.34 mm long, 11.40 \pm 0.34 mm wide, 1.49 \pm 0.03 mm thick) were scanned at 0.05% strain and 6.28 rad/sec (1 Hz), parameters within the linear viscoelastic range of the material, from -80 to 160°C at 5°C/min. The glass transition was determined as the temperature at loss modulus (G'') maximum (Aklonis and MacKnight 1983).

Pressure Rheometry

The dynamic properties of gluten were determined with a rheometer (RPR, Rheometrics, Piscataway, NJ) operating at 200 psi to prevent moisture loss when samples were heated to temperatures >100°C. Gluten disks (20 mm diameter, 2 mm thick) were placed between parallel plates, allowed to relax for 15 min, and then scanned from 40 to 190°C at 5°C/min. Apart from the sample with 10% moisture, which was scanned at a frequency of 0.75 rad/sec due to sensitivity limits of the machine, mechanical spectra were recorded at 0.6% strain and 6.28 rad/sec (1 Hz).

Moisture content was determined by vacuum drying at 100°C and 25 mm Hg for 6 hr (AACC 2000). Reported values are means of at least triplicate determinations unless otherwise stated.

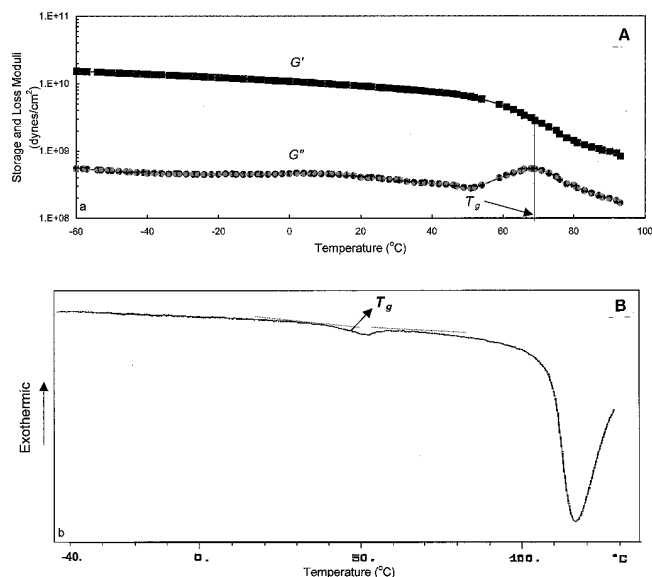


Fig. 1. Typical thermograms of gluten from mechanical spectrometry at 7% moisture (A) and differential scanning calorimetry at 12.5% moisture (B).

Glass Transition of Gluten

Typical thermograms of wheat gluten, obtained by mechanical spectrometry and DSC, are shown in Fig. 1. Gluten exhibited a drop in storage modulus (G'), typical of amorphous polymers, at the glass transition. However, the magnitude of drop in G' (1 decade) was smaller than that typically reported for synthetic polymers (3 decades) (Kalichevsky et al 1993). This smaller than expected drop in G' could be due to the intrinsic cross-links in gluten. We do not subscribe to the view presented by Weegels et al (1994) that heat-induced cross-links are possible at the glass transition temperature because there is not enough mobility for self-diffusion. However, it is possible that the lightly cross-linked network is too elastically inhibited for the typical drop of 3 decades observed in uncross-linked synthetic polymers. Similar findings have been reported elsewhere for gluten (Pouplin et al 1999) and its subfractions (Cocero and Kokini 1991; de Graaf et al 1993). DSC thermograms displayed an initial slope (dH/dT) followed by a higher slope past the glass transition, characteristic of a glass transition temperature, where an inflection in the heat capacity ($C_p = dH/dT$) is expected. The glass transition was also accompanied by a small endothermic transition, which could have been a low temperature relaxation needed for the network to gain the mobility to undergo glass transition. We then observed a large endothermic peak with a minimum at >100°C (Fig. 1). The small endothermic shift moved to lower temperatures and showed less overlap with the large endothermic peak at higher moisture contents, which is consistent with a glass transition (Kokini et al 1995). The large endothermic peak is due to water evaporation from the DSC pan and loss of volatiles formed during heating and to cross-linking reactions in gluten (Sartor and Johari 1996; Ferrari and Johari 1997). Rescanning of samples that have been heated and cooled did not eliminate the glassy state relaxation-related endothermic peak. Rather, the curve was flat in that region, presumably due to the formation of network structures on heating as reported by Sartor and Johari (1996). At moisture contents <5%, the glass transition was not resolved from the larger endothermic peak, possibly due to simultaneous water loss and cross-linking reactions brought about by the decreased mobility of gluten polymers; no unambiguous determination of glass transition temperatures could be made. Difficulties in determining the glass transition temperatures of biopolymers with low moisture contents by standard DSC procedures have been reported (Kalichevsky et al 1992; Noel et al 1995; Bell and Touma 1996). Changes in storage modulus (G') of gluten strips at different moisture contents are shown in Fig. 2. The glass transition temperatures of gluten determined by DSC and mechanical spectrometry were superimposed in the range where data could be obtained in spite of the differences in measured properties and the timeframes of events being measured (Fig. 3). This

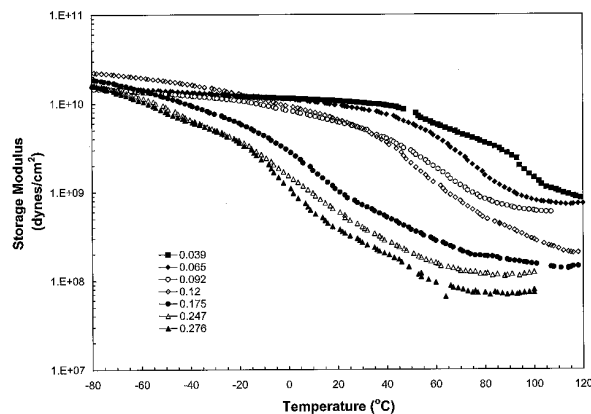


Fig. 2. Storage moduli of gluten at different moisture contents (strain 0.05%; frequency 6.28 rad/sec).

finding lends further support to the reported correspondence of T_g values measured by DSC and mechanical spectrometry (Cocero and Kokini 1991; Kalichevsky et al 1992; de Graaf et al 1993; Chenan and Chinachoti 1996; Gontard and Ring 1996).

The glass transition temperature decreased with increased moisture contents, consistent with the significant plasticizing effect of water on gluten and other proteins (Slade and Levine 1995). However, the T_g remained constant at -20°C for samples with moisture contents of $\geq 24\%$, indicating the development of a maximally freeze-concentrated system under these conditions. The T_g' is the glass transition temperature of the maximally freeze-concentrated gluten matrix. T_g' values were lower than the values (-5 to -10°C) reported by Levine and Slade (1990) and Chenan and Chinachoti (1996) and lower than the values we expected (-11 to -13°C) for high molecular weight proteins (Brake and Fennema 1999).

The glass transition temperature of dry gluten could not be determined by mechanical spectrometry, especially because samples equilibrated over P_2O_5 sorbed variable amounts of water (1.8–3.0%) during loading and equilibration in the mechanical spectrometer oven. These limitations, coupled with the aforementioned inability to locate glass transition temperatures of low moisture samples by DSC, precluded the determination of T_g of dry gluten. Accordingly,

the T_g of dry gluten was estimated by the Gordon-Taylor equation (Gordon and Taylor 1952) because of its reported adequacy to predict the glass transition temperatures of binary mixtures of water and gluten, gliadin, glutenin, soy globulins, and zein proteins (Kalichevsky et al 1992; de Graaf et al 1993; Noel et al 1995; Kokini et al 1995; Madeka and Kokini 1996; Morales-Diaz and Kokini 1998). According to the Gordon-Taylor equation, the T_g of binary mixtures of gluten and water is expressed as $T_g = (w_1T_{g1} + kw_2T_{g2})/(w_1 + kw_2)$ where T_{g1} and T_{g2} are the glass transition temperatures of dry gluten and water respectively, w_1 and w_2 are the weight fractions of gluten and water, respectively, and k is a constant representing the strength of the gluten-water interaction.

Based on a value of 139.15 K for T_g of water (Johari et al 1987), a T_g of $127.6 \pm 4.1^\circ\text{C}$ for dry gluten and a k value of 3.9 ± 0.24 were obtained with a coefficient of determination (r^2) of 0.98 between the measured and predicted T_g values of gluten-water mixtures. The T_g value of dry gluten was lower than the best-fit value (162°C) obtained by simulating the thermal behavior of gluten-water mixtures using the Gordon-Taylor equation (Kalichevsky et al 1992), the value (150°C) measured by DSC (Cherian and Chinachoti 1996) or the value (187°C) obtained by dynamic mechanical thermal analysis (Pouplin et al 1999). These differences could be attributed to differences in the composition of the materials, sample preparation, thermal history of samples, or conditions of measurement.

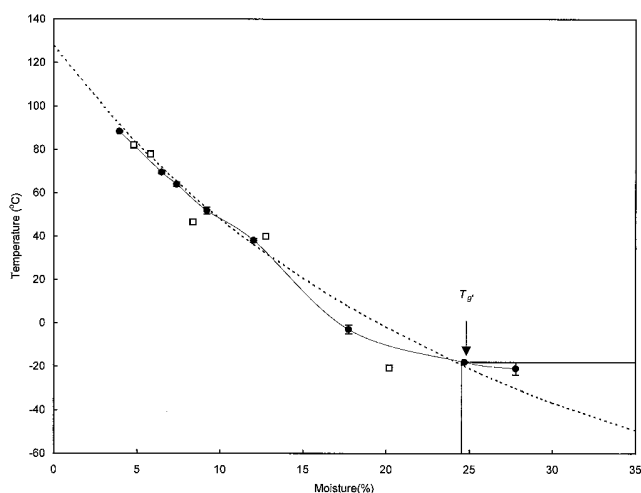


Fig. 3. Glass transition temperatures of gluten at different moisture contents from mechanical spectrometry (●) and differential scanning calorimetry (□). Dotted line: Gordon-Taylor fit for glass transition temperatures.

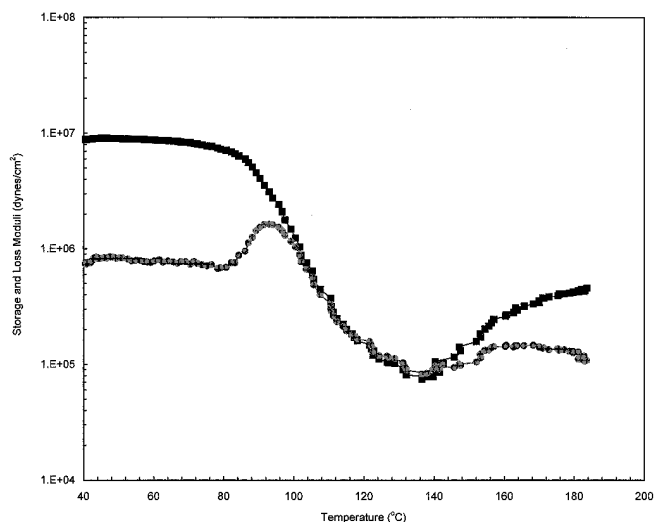


Fig. 4. Temperature sweep of gluten at 10% moisture (strain 0.6%, frequency 0.75 rad/sec, pressure 200 psi).

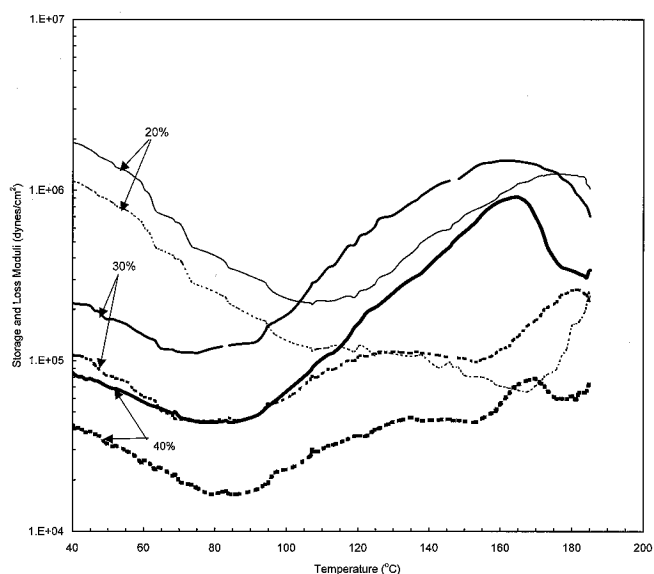


Fig. 5. Temperature sweeps of gluten at 20, 30, and 40% moisture (strain 0.6%; frequency 6.28 rad/sec; pressure 200 psi).

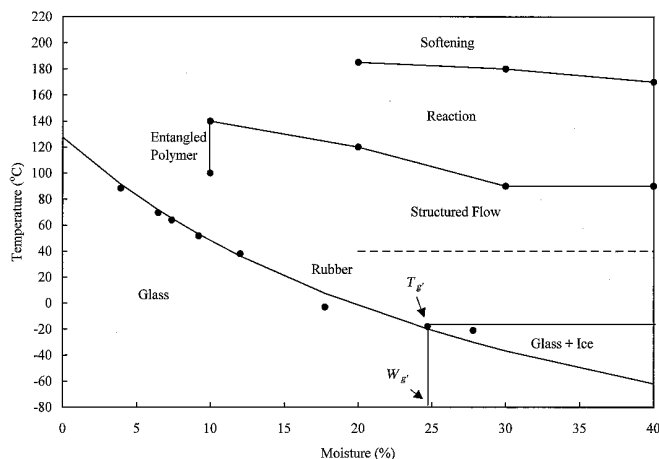


Fig. 6. State diagram for gluten.

Characterization of Gluten States

No change in the magnitudes of G' and G'' (with G' being 1 decade higher than G'') was observed for gluten with 10% moisture at 40–80°C. A decrease in G' and an increase in G'' were observed when the temperature was >80°C, with G'' exhibiting a maximum similar to that displayed by materials undergoing a glass transition at 90°C (Fig. 4). This apparent glass transition was ≈35–40°C higher than the corresponding T_g determined by mechanical spectrometry or DSC. Glass transition temperatures have not been investigated by pressure rheometry due to the limits of sensitivity of existing machines, especially as strains <0.5% and torques corresponding to $G' \geq 10^8$ dynes/cm² cannot be accessed. Therefore, inferences and comparison of glass transition-like transformations observed by pressure rheometry with those determined by established procedures must be viewed with caution.

The magnitudes of G' and G'' for 10% moisture gluten decreased sharply and were almost identical at 95–140°C. This behavior is indicative of an entangled polymer flow. With further increase in temperature, G' increased until the upper limit of the investigated temperature range was reached (190°C), while G'' increased between 140 and 160°C and slowly decreased thereafter (Fig. 4). This pattern of change in the magnitudes of G' and G'' is likely due to molecular aggregation and cross-linking reactions mediated by the increased mobility of the polymer chains. Heating of gluten with moisture contents <13% results in an increase in hydrophobic interactions and a decrease in SDS extractability of glutenin aggregates (Weegels et al 1994).

The magnitude of G' and G'' for 30% moisture gluten decreased at 40–70°C and showed little change with further heating to 93°C (Fig. 5). The magnitude of G' was 2.1–2.3× that of G'' at 40–93°C. Materials exhibiting such behavior have been described as experiencing a structured or rubbery flow (Kokini et al 1995; Morales-Diaz and Kokini 1998). The storage modulus increased sharply as the material was heated to >93°C, indicating the formation of cross-links presumably by disulfide bonds and the development of a network structure. This finding is consistent with the reported increase in G' of gluten when heated to >87–91°C (Attenburrow et al 1990). Furthermore, an increase in the proportion of SS bonds on heating of gluten has been reported (Schofield et al 1983; Weegels et al 1994). The storage modulus increased to a maximum of 1.5×10^6 dynes/cm² at 157°C, while G'' increased slowly from 93 to 120°C and remained almost constant at 120–140°C. The maximum G' coupled with the minimum observed for G'' at 157°C indicates the development of a network structure with maximum cross-link density. The storage modulus did not change at 157–165°C and decreased thereafter, while G'' increased to a maximum at 181°C, indicating devitrification, degradation, and softening of the network structure. The pattern of change in G' and G'' indicates that after structured or rubbery flow up to 93°C, 30% moisture gluten undergoes networking reactions at 93–181°C, with maximum structure buildup between 157 and 165°C, followed by devitrification, degradation, and softening at ≥181°C (Fig. 5).

Similar patterns were observed for gluten with 20 and 40% moisture, although thermal transitions occurred at temperatures different from those for 30% moisture gluten (Fig. 5). Networking reactions commenced at 120°C in gluten with 20% moisture, as compared with 93°C for 30 and 40% moisture gluten. Moreover, devitrification, degradation, and softening of the network structure occurred at 184, 181, and 170°C in 20, 30, and 40% moisture glutes, respectively. These findings are consistent with the role of water as mobility catalyzer (Levine and Slade 1990), allowing thermal transitions to occur at lower temperatures in materials with correspondingly higher water contents.

The buildup of maximum strength in gluten networks has been attributed to cross-linking reactions between polymer chains and the progressive increase in the matrix viscosity brought about by the greater interactions between the newly formed higher molecular weight structures (Belton 1999).

State Diagram

The states of gluten, as inferred from glass transition measurements and temperature sweeps, are summarized in a preliminary state diagram (Fig. 6). Gluten was significantly plasticized by water (Slade et al 1989; Levine and Slade 1990) in accord with its hydrophilic nature. The glassy region and rubbery domains are located below and above the glass transition line, respectively. The T_g of maximally freeze-concentrated gluten (T_g') (Levine and Slade 1990) and the corresponding weight fraction (C_g') of gluten are –20°C and 76%, respectively. At 10% moisture, gluten traverses an entangled polymer flow region at 92–140°C and enters the reaction zone thereafter, where a network structure is developed through disulfide bond formation. The dashed line marks a hypothetical transition from the rubbery to the structured flow region. This was not observed under the conditions of measurement presumably because it takes place at <40°C. Increasing water levels allowed gluten to cross-link at lower temperatures. Network structure started to develop at 120°C at 20% moisture and at 93°C when water was present at the levels of 30 and 40%. Softening of the network structure was not observed in 10% moisture gluten in the investigated temperature range but decreased with increased water levels in samples with higher moisture contents (20, 30 and 40% moisture glutes softened at 184, 181 and 170°C, respectively). At moisture contents of 20–40%, gliadin and glutenin undergo cross-linking reactions at 65–70°C and devitrification or softening of the vitrified network at 135–145°C, respectively (Kokini et al 1994, 1995). The occurrence of the aforementioned thermal transitions for gluten subfractions at lower temperatures, as compared with those for gluten, could be attributed to the higher mobility of gliadin chains (Kokini et al 1994). The significant contribution of hydrophobic interactions could be attributed to structure buildup in glutenin (Tsiami et al 1997a,b). The onset temperature of the reaction zone and the temperature at which softening is observed may correspond with the gelling temperature of the nonreacted material (T_{gel}) and the devitrification temperature of the vitrified network (T_{veg}) respectively, in the time-temperature transformation diagram previously proposed for gluten (Levine and Slade 1990).

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