

Rheological Properties of Vital Wheat Gluten Suspensions

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ABSTRACT

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Flour and doughs represent rheologically complex materials whose properties are dependent on many factors including processing conditions. To avoid some of the problems associated with the rheological characterization of dough, we have initiated a study focused on the rheological properties of one of the major components of dough, vital wheat gluten. Suspensions of vital wheat gluten were prepared with concentrations of 225–325 mg/mL. The moduli of the gluten suspensions was 0.2 Pa at 225 mg/mL to 37 Pa at 325 mg/mL. At <250 mg/mL, the gluten suspensions exhibited fluidlike behavior. The crossover frequency, ($G'[\omega] = G''[\omega]$)

shifted slightly from 0.5 rad/sec at 225 mg/mL to 0.9 rad/sec at 250 mg/mL. At >300 mg/mL, the gluten suspensions exhibited solidlike behavior. The crossover frequencies were independent of concentration and equal to 100 rad/sec. At <250 mg/mL, the high-frequency behavior of moduli were proportional to $\omega^{3/4}$, as expected for a semiflexible coil. At >300 mg/mL, the high-frequency behavior of moduli were proportional to $\omega^{1/2}$, indicating a flexible coil. These results suggest vital wheat gluten suspensions undergo a structural change between 250 and 300 mg/mL.

The breadmaking quality of wheat flour depends on both the quantity and quality of its proteins, especially gluten. Gluten is viscoelastic (Hibberd and Parker 1975; Attenburrow et al 1990), and is composed of ≈80% (db) proteins. The unique structure of gluten provides many special physical properties suitable for foods such as bread, noodles, etc., as well as nonfood industrial uses including films and biodegradable plastics (Cuq et al 1998).

Gluten is composed of two major kinds of proteins, glutenin and gliadin. Gluten has a high percentage of amide nitrogen mainly from glutamine, a high percentage of proline, intra- and intermolecular disulfide bonding, and many amino acids with hydrophobic side chains. Glutamine is rich in gliadins (≈35%). Proline makes sharp bends in the molecules and disrupts protein secondary structures. Intra- and intermolecular disulfide bonding caused by cysteine can be found mainly in glutenins; much of the intramolecular disulfide bonding is in gliadins.

The rheological properties of flour dough are important to the good quality of bakery products. However, much work in flour dough rheology toward understanding the mechanism of controlling flow and deformation remains to be done. The gluten complex is a major contributor to the physical properties of flour dough. The rheological properties of gluten doughs constitute a rich field of study on the complex behavior of this system (Schofield et al 1983, 1984; Hosney et al 1987; Dreese et al 1988a,b; Brockway et al 1989; Eliasson and Lundh 1989; Attenburrow et al 1990; He and Hosney 1990; Khatkar et al 1995; Petrofsky and Hosney 1995; Bagley et al 1998; Redl et al 1998; Miller and Hosney 1999). Attenburrow et al (1990) reported that the oscillatory storage modulus G' (elasticity) of gluten dough is ≈2,000–8,000 Pa. The G' of a gluten dough is temperature-dependent, decreasing when heated to ≈60°C, then increasing at higher temperatures because of gelatinization of a small amount of starch, and then increasing rapidly at ≈90°C, probably due to formation of new cross-links (Attenburrow et al 1990). The rheological properties of gluten dough also depend on water content. The value of G' decreases with increasing moisture

content (Dreese et al 1988b; Attenburrow et al 1990). Dreese et al reported that G' of gluten dough was 50–25,000 Pa, depending on frequency and water content.

Processing conditions including mixing procedure, mixing time, and rest time also have large effects on measured rheological properties of gluten doughs. Variations in any of these parameters can result in gluten doughs with widely varying rheological properties. The complex nature of gluten doughs makes it difficult to establish a baseline of rheological parameters for dough.

The present study avoided these difficulties by examining the linear dynamic rheological properties of suspensions of one of the major components of dough, vital wheat gluten. By accumulating data, we anticipated that we could establish a model to describe basic rheological properties of doughs in the near future.

MATERIALS AND METHODS

Materials

The vital wheat gluten samples used in this study were donated by Ody Maningat of Midwest Grain Products, Inc. (Atchison, KS). They contained a minimum of 75% of protein ($N \times 5.7$), 1.0–2.0% ash, 5.0–8.0% moisture, and 1.0–2.0% fat, as well as a maximum of 1.0% fiber. The materials were used as received.

Sample Preparation

Gluten was suspended in a 0.05M sodium phosphate, pH 7.0, 25°C, solution containing 3M urea through extensive mixing using a stirrer (Wu and Dimler 1964; Ewart 1980; Bietz 1984; Huebner and Bietz 1985). Observation by microscope showed gluten was well dispersed, and no sedimentation was observed for two weeks after the sample preparation. Samples were stored at 4°C and used within 10 days of preparation to avoid sample degradation. At least two suspension samples were made for each concentration for the measurements.

Microscopy

Gluten suspensions were observed directly with a Zeiss Axioskop photomicroscope using phase-contrast optics and a 100X oil-immersion objective.

Rheological Measurements

Rheological properties of gluten suspensions were measured with a strain-controlled fluids rheometer (Rheometrics ARES series IV) using a 50-mm diameter cone-plate geometry. The angle of the cone was 0.04 radians. The sample chamber was enclosed in a humidity chamber to prevent evaporation of solution. The temperature was controlled at $25 \pm 0.1^\circ\text{C}$ in the experiment chamber using a water circulation system. Before measuring dynamic rheological parameters, a strain-sweep experiment was conducted to ensure

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the experiments were conducted in linear viscoelastic range. Linear viscoelasticity indicates that the measured parameters are independent of shear strains. All measured materials in this study were in the linear range at <8% strain. Stress relaxation experiments were also performed in the linear viscoelastic range. These experiments measured the stress relaxation with the time after the material is subject to a step increase in strain. Small-amplitude oscillatory shear experiments (shear strain = 1%) were conducted over a frequency (ω) range of 0.001–100 rad/sec, yielding the shear storage G' and loss G'' moduli. The G' represents the nondissipative component of mechanical properties. The elastic or rubberlike behavior is suggested if the G' spectrum is independent of frequency and greater than G'' over a certain range of frequency. The G'' represents the dissipative component of the mechanical properties and is characteristic of viscous flow. The phase shift ($\delta = \tan^{-1} [G''/G']$) indicates whether a material is solid ($\delta = 0$), or liquid ($\delta = 90^\circ$) or something in between. The plateau modulus indicates that the stress relaxation measured modulus is nearly constant over a measured range of time. Each measurement was repeated at least twice with different samples. The relative errors were all within the range of $\pm 12\%$.

RESULTS

Concentration Dependence of Gluten Viscoelastic Properties

The linear dynamic frequency sweep results for four concentrations of gluten suspensions are shown in Fig. 1. The G' and G'' values for the gluten suspensions were dependent on the oscillation frequency, especially at higher frequencies. Values of these moduli were lower than those of gluten doughs (Schofield et al 1983, 1984; Hosney et al 1987; Dreese et al 1988a,b; Brockway et al 1989; Eliasson and Lundh 1989; Attenburrow et al 1990; He and Hosney 1990; Khatkar et al 1995; Petrofsky and Hosney 1995; Redl et al 1998, Miller and Hosney 1999). Gluten doughs are more concentrated and more complicated than gluten suspensions. So it might not be appropriate to compare them directly. However, the lower values of gluten suspensions moduli may reflect the basic properties of gluten.

Both G' and G'' of the gluten suspensions depended strongly on concentration. The G' and G'' values were frequency-independent only at low frequencies. At 0.01 rad/sec, G' of the 225 mg/mL gluten suspension was 0.18 Pa; while G' of the 325 mg/mL gluten suspension increased to 37 Pa (Figs. 1 and 2). Thus, within this

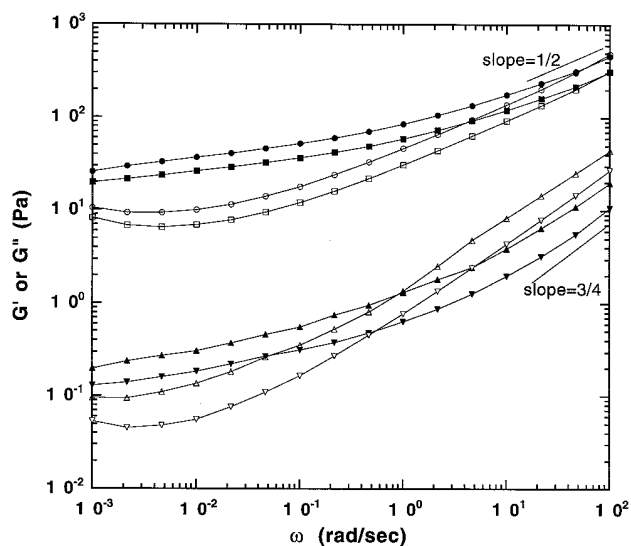


Fig. 1. Dynamic frequency-dependence moduli of different concentrations of gluten suspensions. \blacktriangledown = 225 mg/mL, \blacktriangle = 250 mg/mL, \blacksquare = 300 mg/mL, \bullet = 325 mg/mL; filled symbols G' , opened symbols G'' .

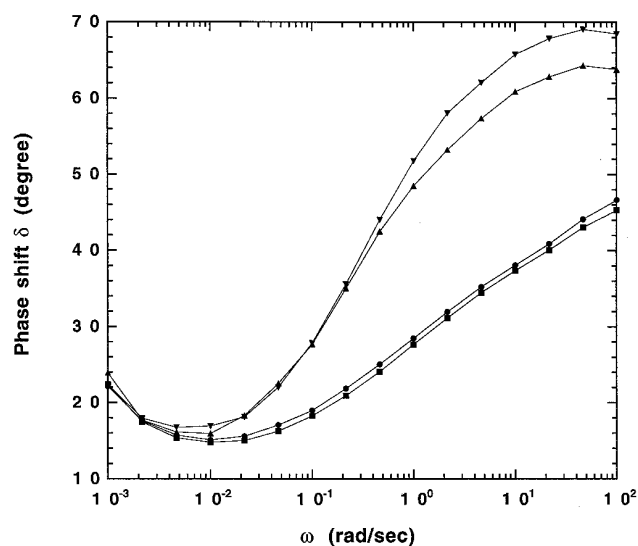


Fig. 3. Frequency-dependence phase shift of different concentrations of gluten suspensions. \blacktriangledown = 225 mg/mL, \blacktriangle = 250 mg/mL, \blacksquare = 300 mg/mL, \bullet = 325 mg/mL.

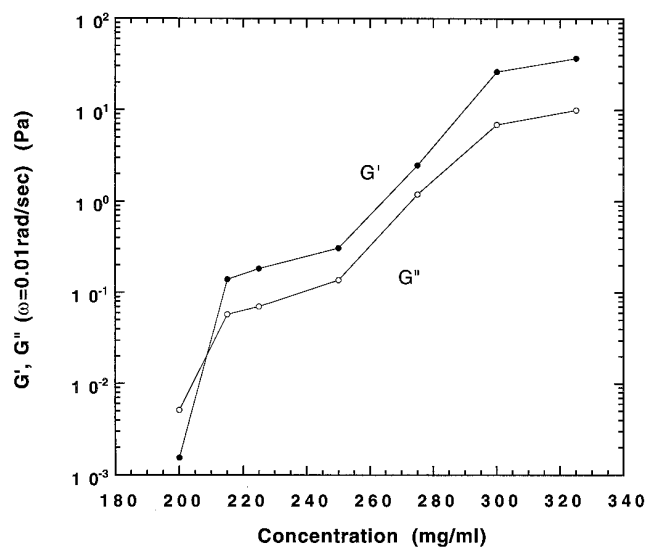


Fig. 2. Frequency-independent dynamic moduli vs. gluten suspension concentrations. Filled symbols G' , opened symbols G'' .

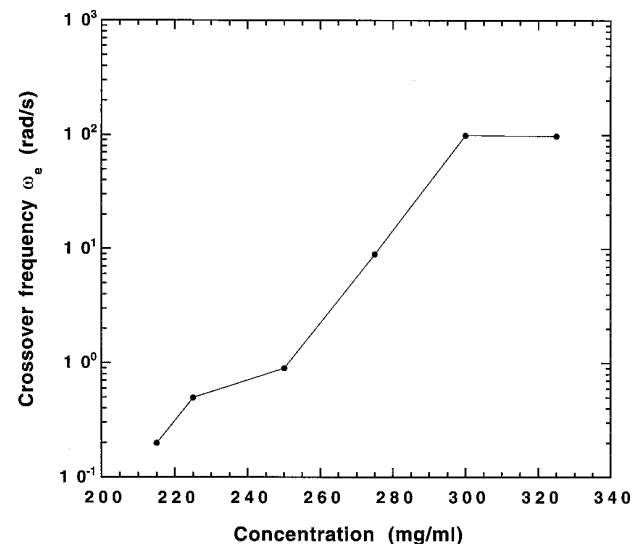


Fig. 4. G' and G'' crossover frequency ($G' = G''$ at ω_c) vs. gluten suspension concentrations.

narrow concentration range, frequency-independent modulus increased by more than 200× (Fig. 2). In addition, the viscoelastic behavior of gluten was also distinct for different suspension concentrations. At <250 mg/mL, gluten suspension properties were more fluidlike, according to linear viscoelastic theory (Ferry 1980). The moduli were strongly frequency-dependent. Phase shifts ranged from 23° at 10⁻³ rad/sec to 65° at 100 rad/sec (Fig. 3). The crossover frequencies were 0.5 rad/sec and 0.9 rad/sec for the 225 and 250 mg/mL gluten suspensions, respectively (Fig. 1 and 4). So, at <250 mg/mL, the crossover frequencies were not significantly changed with the increased concentration. However, the shear moduli versus radial frequency curves at higher gluten concentrations (≥300 mg/mL) had a longer frequency-independent plateau, which indicates more solidlike behavior (Ferry 1980) (Fig. 1). The G' was higher than G'' over most of the measured frequency range for the gluten suspensions at ≥300 mg/mL. Phase shifts were from 23° at 10⁻³ rad/sec to 45° at 100 rad/sec (Fig. 3). The G' and G'' crossover frequency was ≈100 rad/sec for both 300 and 325 mg/mL gluten suspensions. For 250–300 mg/mL gluten suspensions, the crossover frequencies shifted sharply from <1 rad/sec to ≈100 rad/sec, indicating the property change.

Morphology Study

Microscopic examination of resulting gluten suspensions revealed a uniform dispersion of particles of <40 μm (Fig. 5). These observations did not show much difference between 250 and 300 mg/mL gluten suspensions. Visibly, however, they were clearly distinct from each other. The 250 mg/mL sample was fluidlike, but the 300 mg/mL sample was more solidlike. These results suggested that the change might occur at the molecular level and could not be observed by microscopy.

Stress Relaxation Study

Stress relaxation measurements (Fig. 6) were conducted on the gluten suspensions. Dashed lines in the graphs showed the mul-

tiplex relaxation time fitted with the Maxwell model (Macosko 1994). The model may be expressed as:

$$G(t) = G_e + \sum G_i \exp(-t/\tau_i) \quad (1)$$

where $G(t)$ is the relaxation modulus, G_e is the plateau modulus, G_i is the i^{th} weighting constant, t is the time, and τ_i is the i^{th} relaxation time. The KaleidaGraph program (Synergy Software, Reading, PA) was used to fit the relaxation time with a five-constant Maxwell model. The results for the 250 and 300 mg/mL samples are shown in Table I. Fitted relaxation times for the 250 mg/mL sample were 0.02–200 sec. The relaxation spectrum showed that the plateau was short; the plateau modulus was 0.04 Pa (Fig. 6A). For the 300 mg/mL gluten suspension, relaxation times were 0.1–1,000 sec, five times longer than for the 250 mg/mL gluten suspension. The relaxation moduli for 300 mg/mL gluten suspension were much greater than for 250 mg/mL gluten; 300 mg/mL gluten had a longer plateau. The plateau modulus of the 300 mg/mL gluten was 8 Pa (Fig. 6B), 200× higher than for the 250 mg/mL gluten. This indicated that 300 mg/mL gluten suspension was more strongly cross-linked or entangled than was the 250 mg/mL gluten suspension. This result is also characteristic of a viscoelastic solid for the ≥300 mg/mL gluten suspensions.

High-Frequency Moduli Behavior

The high-frequency moduli behavior of <250 mg/mL gluten suspensions was also very different from those of the ≥300 mg/mL gluten suspensions. The high-frequency moduli behavior was determined by log-log complex moduli $|G^*|$ ($|G^*| = (G'^2 + G''^2)^{1/2}$) curve slope at frequencies >40 rad/sec. High-frequency moduli for 225 and 250 mg/mL gluten suspensions were proportional to $\omega^{3/4}$ (Figs. 1 and 7), indicative of a semiflexible polymer (Morse 1998a–c). However, high-frequency moduli for 300 and 325 mg/mL gluten suspensions were proportional to $\omega^{1/2}$, indicating the material with flexible coil-like behavior (Ferry 1980). For ≤ 250 mg/mL gluten

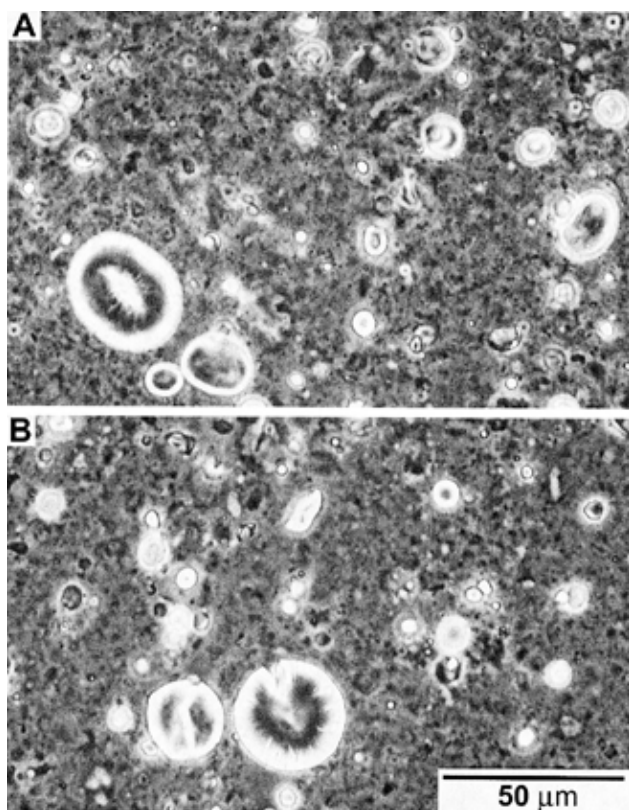


Fig. 5. Phase contrast light micrographs of gluten suspensions: 250 mg/mL (A) and 300 mg/mL (B).

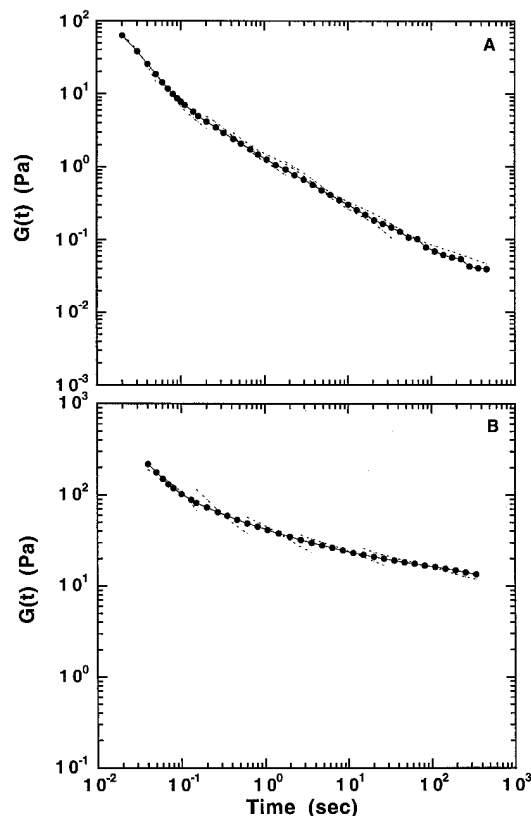


Fig. 6. Stress relaxation experiments and five-constant Maxwell model simulation for gluten suspension of 250 mg/mL (A) and 300 mg/mL (B). Solid line: experimental data; dashed lines: Maxwell model.

suspensions, log-log moduli curve slopes at high frequencies (exponents) were all ≈ 0.75 ; for ≥ 300 mg/mL gluten suspensions, exponents were ≈ 0.5 (Fig. 7). The hypothesis of semiflexible or flexible behavior was based on the Doi-Edwards theory, which is applicable to monodisperse solutions (Doi and Edwards 1986). This model may not apply directly to our gluten suspensions. However, information of high-frequency moduli difference implied the difference of polymer chain behavior.

Thus, when gluten suspension concentration increases only 20% (from 250 to 300 mg/mL), its viscoelastic properties shift sharply from a viscoelastic fluid to viscoelastic solid. The flexibility of the chains change from being semiflexible to flexible, and the plateau moduli display a dramatic increase. These results suggest that vital wheat gluten suspensions undergo a major structural change between 250 and 300 mg/mL.

DISCUSSION

Dreese et al (1988b) reported that G' of gluten dough increased with the water content decreased at 46–61% moisture. But the moduli curve shapes and phase shifts were identical for the different gluten doughs with different moistures. The gluten suspensions used at this study had more water content. So it is not appropriate to directly compare them with each other. The tendency of the moduli to change with the concentration, as found in this work, was supported the conclusion of Dreese et al (1988b), who found that G' of gluten dough increased as the water content decreased. However, the viscoelastic behavior shift within a narrow concentration range, which was not observed in gluten doughs by Dreese et al (1988b), indicated that there should be a structure change for the gluten suspensions at this concentration range. The viscoelastic nature of gluten suspensions at ≤ 250 and ≥ 300 mg/mL are totally different.

TABLE I
Relaxation Times Fitted by Maxwell Model
for Wheat Gluten Suspensions^a

i	τ_i (sec)	G_i (Pa)	τ_i (sec)	G_i (Pa)
	250 mg/mL	250 mg/mL	300 mg/mL	300 mg/mL
1	0.02	180	0.1	270
2	0.2	9	1	55
3	2	1.8	10	20
4	20	0.35	100	10
5	200	0.07	1000	5

^a i = iteration; G_i = weighting constant; τ_i = relaxation time

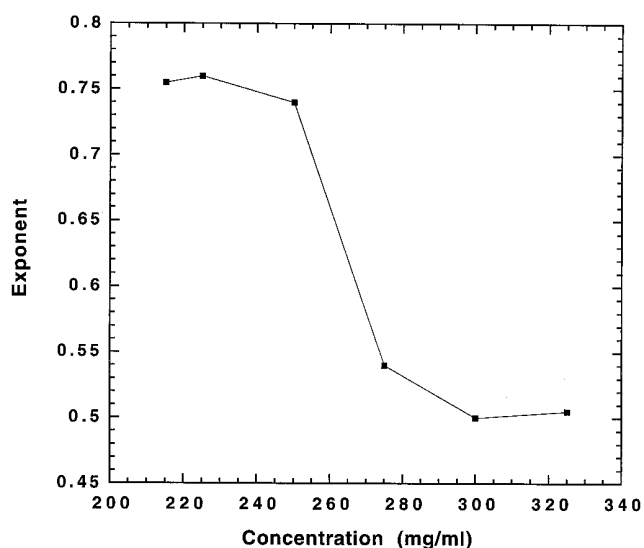


Fig. 7. High-frequency moduli performances of different concentrations, exponent vs. concentration of gluten suspension.

Within this small concentration change, the moduli curve shape, phase shift, stress relaxation behavior, and high-frequency moduli performance all change, which indicates variation in structure. Both 250 and 300 mg/mL gluten suspensions displayed birefringence when observed with a polarizing microscope. But the 300 mg/mL gluten suspension displayed stronger birefringence than at 250 mg/mL (data not shown). This structure change may be due to increased chain-chain interactions and entanglements or cross-linking, etc.

It is difficult to observe these structural differences. The molecular interpretation of rheological properties is not as well understood as other modern biochemical and molecular biology techniques. To relate the rheological parameters to molecular events, it is useful to relate them to the relaxation spectrum (Bird et al 1977). Our relaxation experiments show a dramatic difference between 250 and 300 mg/mL gluten suspensions. The 250 mg/mL gluten suspension has shorter relaxation times (0.02–200 sec). This indicates that the material is a network where the suspended chains can slip past each other in a short time but are not tightly cross-linked or entangled. However, the 300 mg/mL gluten suspension has longer relaxation times (0.1–1000 sec) and a longer plateau. This implies that the material exists as a more entangled network that cannot quickly relax. This structure change may be due to increased inter-chain interactions or cross-linking. If a network is tightly cross-linked chemically, there should not be any relaxation and relaxation time should be infinite. So there must be more physical than chemical chain-chain interactions in the network. Therefore, the more reasonable explanation for the change in structure is that much stronger entanglements occur in ≥ 300 mg/mL gluten suspensions. The high-frequency moduli performances indicate that the ≥ 300 mg/mL gluten suspensions are flexible polymers while the < 250 mg/mL gluten suspensions are semiflexible. At > 300 mg/mL, gluten suspensions probably have more bundles and parallel chains because of stronger chain-chain entanglement. These bundles and parallel chains allow the network to exhibit more flexible behavior. This result, again, supports the earlier conclusion of stronger entanglement in more concentrated gluten suspensions. Additional biochemical and biophysical studies are needed to explore these network structures.

An understanding of the mechanical and rheological properties of wheat gluten is very important for explaining the mechanism of baking, and to guide future research on wheat gluten proteins in food and nonfood applications. Due to the importance of gluten, much research has been conducted on gluten and flour doughs. Dough rheology depends on many factors. Variation in water content, mixing procedure, mixing time, and other factors may lead to gluten doughs with widely varying viscoelastic properties, complicating the investigation of molecular factors affecting the rheological properties of gluten. To better understand the basic rheology of gluten without the complications of dough systems, we studied basic viscoelastic properties in gluten suspensions. The gluten suspensions used in this work have no hydrogen bonding because of the urea in the buffer. So the protein chain-chain interactions and disulfide bond interaction and cross-linking contribute mainly to the viscoelastic properties. Combining further model study and biochemical and biophysical structure work with our results could help us to gain more insight into dough structure and rheology.

CONCLUSIONS

Gluten dispersed in a buffer containing 3M urea exhibits viscoelastic properties that depend strongly on gluten concentration. At < 250 mg/mL, gluten suspensions show viscoelastic liquid properties; high frequency moduli behavior indicates that molecules exist as semiflexible polymers. At > 300 mg/mL, however, gluten suspensions display more viscoelastic solid properties, and high-frequency moduli performance indicates that they are flexible polymers. These results indicate that there is much stronger entanglement of gluten protein chains in ≥ 300 mg/mL suspensions.

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