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Viscoelastic properties of wheat gliadin and glutenin suspensions ☆

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Abstract

Linear and non-linear rheological properties of wheat gliadin and glutenin suspensions were investigated at various concentrations. Linear dynamic viscoelastic properties for both gliadin and glutenin were strongly dependent on concentration. For gliadins, the storage moduli (G'), loss moduli (G''), and phase shifts dramatically changed within a narrow concentration range, indicating that gliadin suspension properties changed from viscous to viscoelastic. Glutenins exhibited viscoelastic solid behaviour at all measured concentrations. The non-linear shear viscoelastic properties of gliadin and glutenin also depended on concentration. Viscosities of gliadins displayed shear-thinning behaviour; viscosities for glutenins showed shear-thickening behaviour at low shear rates, and shear-thinning behaviour at higher shear rates. Our results indicate that gliadin's structure in suspension changes over a small concentration range, and suggest that gliadin is important in adjusting and controlling gluten's viscoelastic behaviour, and not only as a diluent of gluten's functional properties.
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Keywords: Gliadin; Glutenin; Gluten; Wheat proteins; Rheology; Viscoelastic properties

1. Introduction

It has long been known that the breadmaking quality of wheat flour depends on both the quantity and quality of its gluten proteins. In addition, wheat gluten makes possible many non-food applications, such as gluten-based films and molded biodegradable plastics (Cuq, Gontard, & Guilbert, 1998).

Gluten is a viscoelastic protein complex, having both elastic and viscous properties (Attenburrow, Barnes, Davies, & Ingman, 1990; Hibberd & Parker, 1975). Gluten contains two major protein types, glutenin and gliadin. It is generally accepted that glutenin contributes mainly to gluten's elastic properties, while gliadin contributes primarily to gluten's viscous properties (Ciaffi, Tozzi, & Lafiandra, 1996; Mills et al., 1990; Wall, 1979). Some reports suggest that the overall function of wheat proteins derives mainly from glutenin, and that gliadin is only as a diluent (Chakraborty & Khan, 1988; Payne, Corfield, & Blackman, 1979; Weegels, Marseille, Bosveld, & Hamer, 1994). Others, however, suggest that gliadin is an important direct contributor to gluten's properties (Hou et al., 1996; Huebner and Bietz, 1993; Hussain & Lukow, 1997; van Lonkhuijsen et al., 1992; Xu et al., 2002).

There have been several investigations of the role of gluten, glutenin, and gliadin in dough rheology (Attenburrow et al., 1990; Cornec, Popineau, & Lefebvre, 1994; Khatkar, Bell, & Schofield, 1995; Redl, Guilbert, & Vergnes, 1998). It is clear that many factors of processing conditions, such as water content, mixing procedure, mixing time, and rest time have large effects on measured rheological properties of doughs. Variations in any of these parameters can result in doughs with widely-varying rheological properties. Due to the complex nature of doughs, it is difficult to establish a baseline for dough rheological parameters (Xu, Bietz, Felker, Carriere, & Wirtz, 2001).

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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To avoid the uncertainties of dough rheology, we studied gluten rheological properties using suspensions (Xu et al., 2001). We here continue this study by examining the viscoelastic properties of wheat gliadin and glutenin in suspension. To elucidate the separate roles and relationships of gliadin and glutenin, the concentration-dependence of their linear dynamic and non-linear shearing rheological properties were studied. We observed a dramatic change in gliadin's viscoelastic properties over a narrow concentration range, indicating the importance of gliadin in gluten functionality.

2. Materials and methods

2.1. Materials

Wheat gliadin and glutenin samples were obtained from Dr. Ody Maningat of Midwest Grain Products, Inc. (Atchison, KS). Wheat gliadin contained a minimum of 80% of protein ($N \times 5.7$), <6.0% ash, and 3.0–6.0% moisture. The received gliadin sample was named G1. Wheat glutenin contained a minimum of 80% of protein ($N \times 5.7$), 2.0% ash, and <8.0% moisture.

2.2. Sample preparation

The received gliadin sample (G1) was purified by 70% ethanol extraction, centrifuged, and supernatant was saved and dried. The dried supernatant was further purified by repeating the above procedure. The purified gliadin was named G2. Gliadin and glutenin samples were suspended in a buffer of 0.05 M sodium phosphate (pH 7.0 at 25 °C) with 3 M urea through extensive mixing by agitation (Xu et al., 2001). Suspended samples were stored at 4 °C and used within five days of preparation to prevent degradation. At least two samples were made at each concentration for measurements.

2.3. SDS–PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) was carried out with a 12% polyacrylamide gel, as described by Laemmli (1970). Samples were analyzed as reduced with dithiothreitol (DTT).

2.4. Rheological measurements

Rheological properties of gliadin and glutenin suspensions were measured with a strain-controlled fluid Rheometric ARES rheometer (TA Instruments, New Castle, DE), using a 50 mm diameter cone and plate geometry. A humidity chamber was used to cover the apparatus to prevent sample evaporation. Temperature was controlled at 25 ± 0.1 °C, using a circulation system. Prior to dynamic rheological parameter measurements, a strain-sweep experiment was conducted to ensure that measurements were in the linear viscoelastic range. Linear viscoelasticity means that measured parameters are independent of shear strains. Small-amplitude oscillatory shear experiments (within the linear range) were conducted over a frequency (ω) range of 0.001–100 rad s^{-1}, yielding the shear storage G' and loss G'' moduli. The storage modulus represents the non-dissipative component of mechanical properties, and is characteristic of elastic properties of the material. Elastic or "rubber-like" behaviour is suggested if the G' spectrum is independent of frequency and greater than the loss modulus over a certain range of frequency. The loss modulus represents the dissipative component of the mechanical properties and is characteristic of viscous flow of the material. The phase shift (δ) is defined by $\delta = \tan^{-1}(G''/G')$, and indicates whether a material is solid ($\delta = 0$), liquid ($\delta = 90°$), or in between ($0 < \delta < 90°$). Non-linear rheological measurements were conducted as steady shear in the range of shear rate of 0.001–1000 s^{-1}. Each measurement was repeated at least twice with different samples.

3. Results and discussion

Fig. 1. displays the SDS–PAGE of gliadin samples G1 and G2, as well as glutenin. Gliadin sample G1 showed huge amounts of gliadin proteins but was contaminated with small amounts of high and low MW glutenins (Fig. 1). After further purification, gliadin G2 exhibited

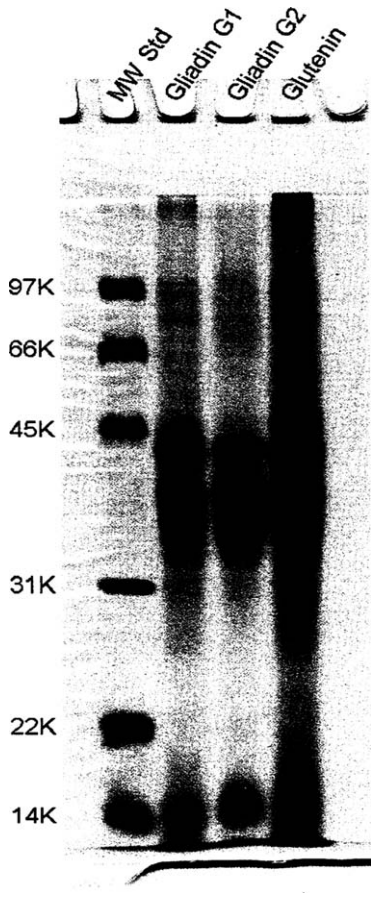

Fig. 1. SDS–PAGE of gliadin and glutenin samples.

almost no glutenin contamination (Fig. 1). Glutenin contained low MW glutenin and was contaminated with other low MW proteins, including gliadin (Fig. 1).

The storage (G') and loss (G'') moduli of four concentrations of gliadin (G1) suspensions are presented in Fig. 2. For 150 and 250 mg/ml gliadin suspensions, G'' was greater than G' over the measured frequency range. The moduli were strongly dependent on frequency, and no plateau was observed. At 150 mg/ml, the storage moduli (G') of gliadin were in the range of 0.0002–2 Pa, the loss moduli (G'') were in the range of 0.0004–4 Pa, and phase shifts were 64°–85°, which correspond with loss tangents of 2.0–10.6 (Fig. 3). These results suggested that gliadin exhibits viscous properties at this concentration, though its properties are not those of a perfectly homogeneous viscous fluid, such as oil or glycerol. From 150 to 250 mg/ml, G'' were still greater than G' over the measured frequencies. Values of the moduli increased with increasing concentration. At 250 mg/ml, G' and G'' were in the range 0.002–15 Pa and 0.006–30 Pa, respectively. The phase shifts decreased to 60°–73° which correspond with loss tangents of 1.8–3.5 (Fig. 3). At 250 mg/ml, even though gliadin suspensions are somewhat viscous, gliadin's heterogeneity increased. At higher concentrations, heterogeneity of gliadin suspensions increased more (Figs. 2 and 3). Storage moduli (G') were higher than loss moduli (G'') at lower frequencies for 300 and 350 mg/ml gliadin suspensions (G1). In addition, viscoelastic behaviours of gliadin at these two concentrations were almost identical, and there was a short plateau of moduli (Fig. 2). G' and G'' ranged from 0.5 to 66 Pa and 0.3 to 99 Pa, respectively, and their phase shifts were 28°–53° which correspond with loss tangents of 0.4–1.5 (Fig. 3). The phase shifts for four concentrations of gliadin suspensions displayed clear differences between $\geqslant$300 mg/ml and $\leqslant$250 mg/ml (Fig. 3). This indicated that at $\geqslant$300 mg/ml, gliadin suspensions deviate more from that of viscous liquid. From 250 to 300 mg/ml, the concentration changes 20%, while properties of gliadin suspensions dramatically increase in viscoelasticity. Because the gliadin (G1) that we used is not pure and there was some glutenin contamination, it is difficult to conclude that the properties change stated above, originated from gliadin. Therefore, we repeated the above experiment using the purified gliadin sample G2. The results for the gliadin G2 were identical to the gliadin G1 suspensions (data not shown), indicating that tiny amounts of glutenin could not affect the properties of a large amount of gliadin. Thus, a reasonable explanation for dramatic properties shift of the gliadin suspensions is that it is due to the gliadin structure itself.

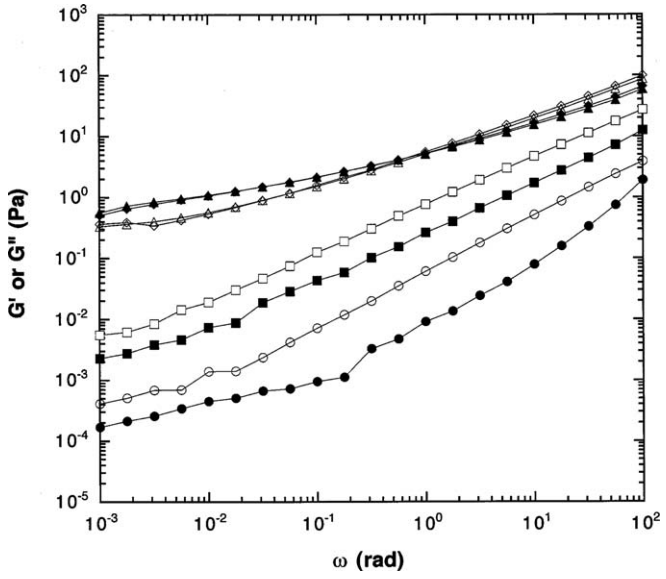

Fig. 2. Dynamic frequency-dependence moduli of different concentrations of gliadin suspensions. Diamond = 150 mg/ml, triangle = 250 mg/ml, square = 300 mg/ml, circle = 350 mg/ml; filled symbols G', opened symbols G''.

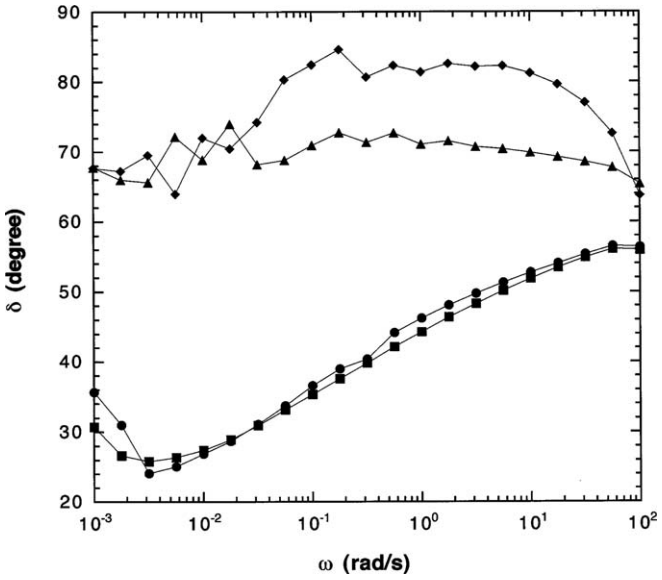

Fig. 3. Frequency-dependence phase shift of different concentrations of gliadin suspensions. Diamond = 150 mg/ml, triangle = 250 mg/ml, square = 300 mg/ml, circle = 350 mg/ml.

Glutenin suspensions showed strong viscoelastic solid behaviour, which is coincident with other scientists' results (Attenburrow et al., 1990; Cornec et al., 1994; Khatkar et al., 1995; Redl et al., 1998). Glutenin suspensions, at four concentrations (Fig. 4), showed parallel shapes for G' and G''; G' were greater than G'' at measured frequencies, and G' had a long plateau over a wide range of oscillation frequencies, indicating an elastic or "rubber-like" behaviour. Phase shifts for four concentrations of glutenin suspensions ranged from 4°–38° which correspond with loss tangents of 0.07–0.8 (Fig. 5). At 0.1 rad s^{-1}, G' of the 100 mg/ml glutenin suspension was 2.5 Pa, while G' of the 150, 200, and 250 mg/ml glutenin suspensions increased to 7.5, 35 and 85 Pa, respectively. Thus, within a narrow concentration range, elastic moduli of glutenin increased more than 30 times. These results suggested that the glutenin becomes increasingly stronger as concentration increases, and exhibits the same viscoelastic solid properties at all measured

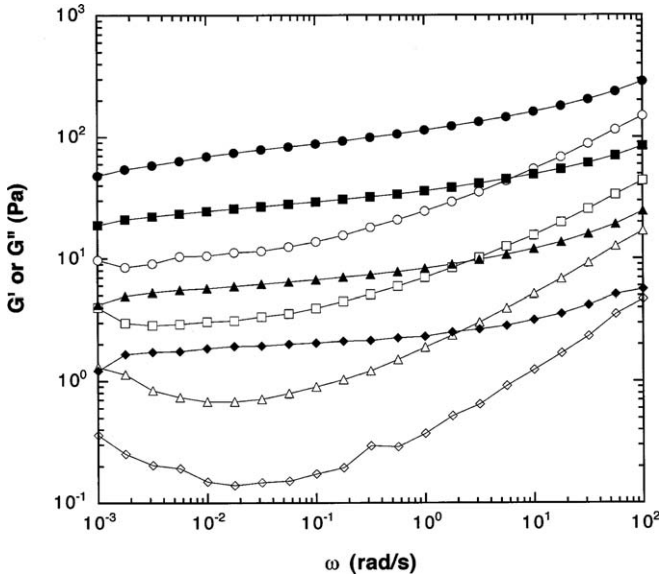

Fig. 4. Dynamic frequency-dependence moduli of different concentrations of glutenin suspensions. Diamond = 100 mg/ml, triangle = 150 mg/ml, square = 200 mg/ml, circle = 250 mg/ml; filled symbols G', opened symbols G''.

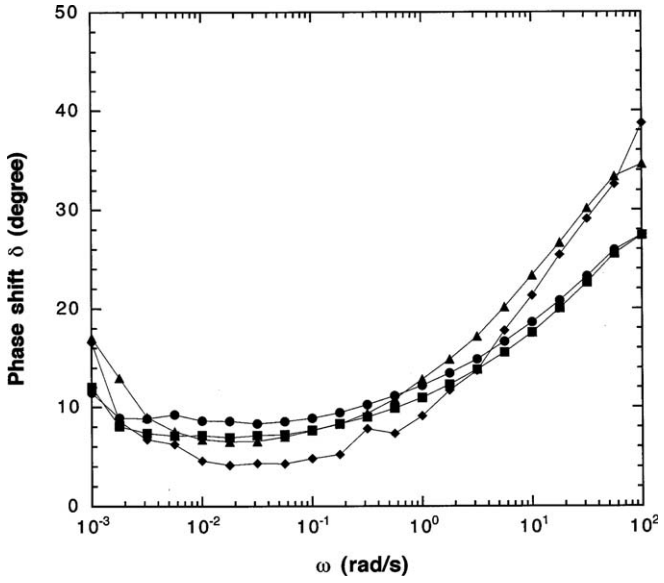

Fig. 5. Frequency-dependence phase shift of different concentrations of glutenin suspensions. Diamond = 100 mg/ml, triangle = 150 mg/ml, square = 200 mg/ml, circle = 250 mg/ml.

concentrations. The glutenin that we used was not pure by the analysis shown above. However, it showed strong viscoelastic solid behaviour, which is consistent with many other scientists' conclusions for glutenin and very different from the behaviour of gliadin obtained above as well as the behaviour of gluten (Xu et al., 2001). Apparently, the contaminated low MW proteins, including some gliadin, had little influence on the properties of glutenin. In addition, we used a mixture of the glutenin and 2% of purified gliadin G2 to repeat the same measurements. No difference was observed between the mixture suspension and the glutenin suspension alone (data not shown). Thus, a little more of the gliadin made the glutenin more contaminated, but did not affect its properties. Therefore, the above obtained results of strong viscoelastic solid behaviour appear to be the glutenin's property.

Previously, we reported that, in a small concentration range, the viscoelastic properties of a gluten suspension shifted sharply from those of a viscoelastic fluid to those of a viscoelastic solid, that chain flexibility changed from semi-flexible to flexible, and that plateau moduli dramatically increased (Xu et al., 2001). That study showed only that gluten exhibited a dramatic rheological properties change, which must be related to structure alteration. But it did not conclude whether the gluten properties shift was in relation to gliadin or glutenin. From the current study, as noted above, this transition is coincident with the rheological property shift of gliadin suspensions. Apparently, changes in viscoelasticity of gluten networks relate to gliadin behaviour change. In addition, our results also agree with those for another study of gliadin suspensions (Xu, Tseng, Carriere, & Wirtz, 2002). By monitoring thermally driven displacements of imbedded microspheres by video fluorescence microscopy (multiple-particle tracking), we quantified microstructural and micromechanical heterogeneities of wheat gliadin suspensions. Heterogeneity of suspensions increased dramatically over a narrow range of gliadin concentrations. Microstructural heterogeneities of a 250 mg/ml gliadin suspension were similar to that of a homogeneous viscous aqueous glycerol solution. Heterogeneities of gliadin suspensions largely increased with the increasing concentrations, showing increased viscoelasticity (Xu et al., 2002), while glutenin always exhibits viscoelastic solid properties, for all the concentrations measured. Therefore, our earlier (Xu et al., 2001, 2002) and present results clearly show that glutenin contributed most of the viscoelastic solid properties to gluten behaviour, while gliadin modified gluten's viscoelastic behaviour by varying from viscous to more viscoelastic. Gliadin, therefore, is not only a diluent, but is important in controlling gluten's viscoelastic properties. This conclusion also agrees with those of some other reports (Hou et al., 1996; Hussain & Lukow, 1997).

The non-linear viscoelastic properties of gliadin and glutenin suspensions are illustrated in Figs. 6 and 7, respectively. Gliadin (G1) suspensions showed shear-thinning behaviour over the entire measured shear rates (Fig. 6). Purified gliadin sample G2 showed the same results as gliadin sample G1 (data not shown). Viscosities were higher at higher gliadin and glutenin concentrations, as expected. Viscosities of glutenin suspensions were different and orders greater than those of gliadin suspensions, and glutenin suspensions exhibited some shear-thickening behaviour at low shear rates (0.001 and 0.02 s^{-1}). Over most of the measured shear rates, glutenin showed shear-thinning behaviour (Fig. 7). The shear-thickening behaviour of glutenin may relate to structural change or rearrangement.

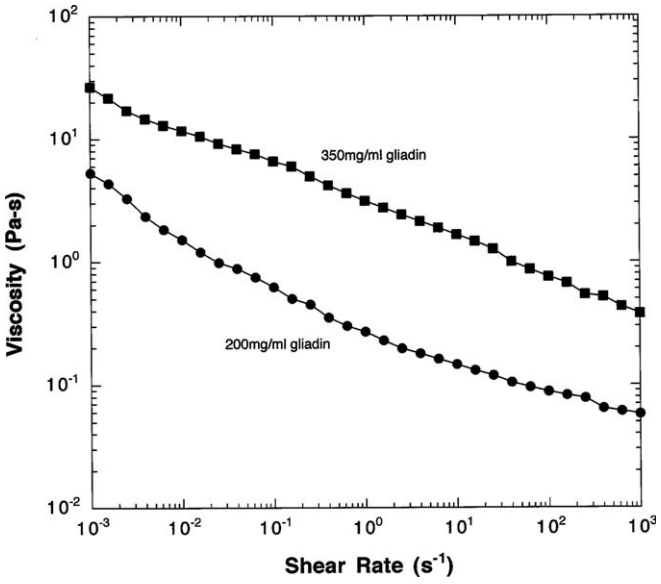

Fig. 6. Non-linear steady shear viscosities for gliadin suspensions. circle = 200 mg/ml, square = 350 mg/ml.

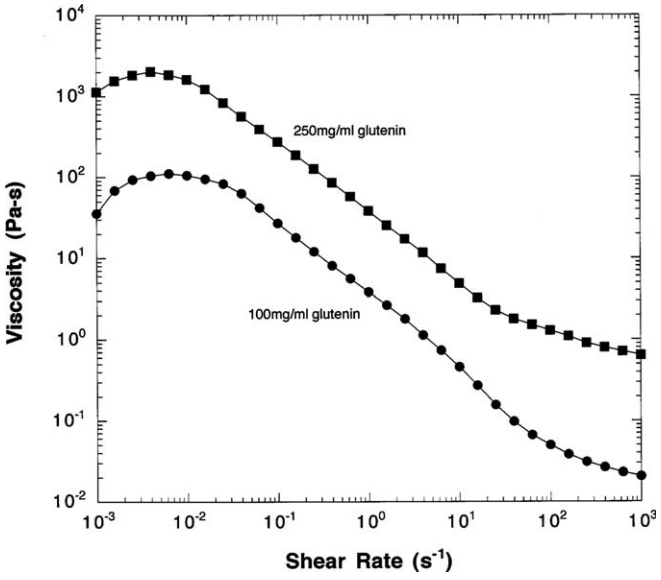

Fig. 7. Non-linear steady shear viscosities for glutenin suspensions. Circle = 100 mg/ml, square = 250 mg/ml.

The mechanism of this shear-thickening behaviour is unknown, and will be further investigated.

4. Conclusion

We investigated rheological properties of gliadin and glutenin suspensions. For both protein fractions, linear and non-linear rheological properties were strongly dependent on concentration. Glutenin exhibited viscoelastic solid behaviour, consistent with results of others. However, we found that gliadin did not function only as a diluent. At, $\leqslant$250 mg/ml gliadin suspensions were mostly viscous, but at $\geqslant$300 mg/ml, gliadin exhibited viscoelasticity. In a very narrow concentration range, gliadin's properties altered dramatically. We conclude that, in dough viscoelasticity, gliadin is important in adjusting gluten's viscoelastic properties.

Acknowledgements

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References

Attenburrow, G., Barnes, D. J., Davies, A. P., & Ingman, S. J. (1990). Rheological properties of wheat gluten. *Journal of Cereal Science, 12*, 1–14.

Chakraborty, K., & Khan, K. (1988). Biochemical and bread-making properties of wheat protein components. II. Reconstitution baking studies of protein fraction from various isolation procedures. *Cereal Chemistry, 65*, 340–344.

Ciaffi, M., Tozzi, L., & Lafiandra, D. (1996). Relationship between flour protein composition determined by size-exclusion high-performance liquid chromatography and dough rheological parameters. *Cereal Chemistry, 73*, 346–351.

Cornec, M., Popineau, Y., & Lefebvre, J. (1994). Characterization of gluten subfractions by SE-HPLC and dynamic rheological analysis in shear. *Journal of Cereal Science, 19*, 131–139.

Cuq, B., Gontard, N., & Guilbert, S. (1998). Proteins as agricultural polymers for packaging production. *Cereal Chemistry, 75*(1), 1–9.

Hibberd, G. E., & Parker, N. S. (1975). Measurement of the fundamental rheological properties of wheat-flour doughs. *Cereal Chemistry, 52*, 1r–23r.

Hou, G., Yamamoto, H., & Ng, P. K. W. (1996). Relationship of quantity of gliadin subgroups of selected U.S. soft wheat flours to rheological and baking properties. *Cereal Chemistry, 73*, 352–357.

Huebner, F. R., & Bietz, J. A. (1993). Improved chromatographic separation and characterization of ethanol-soluble wheat. *Cereal Chemistry, 70*, 506–511.

Hussain, A., & Lukow, O. M. (1997). Influence of gliadin-rich subfractions of glenlea wheat on the mixing characteristics of wheat flour. *Cereal Chemistry, 74*, 791–799.

Khatkar, B. S., Bell, A. E., & Schofield, J. D. (1995). The dynamic rheological properties of glutens and gluten sub-fractions from wheats of good and poor bread making quality. *Journal of Cereal Science, 22*, 29–44.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

Mills, E. N. C., Burgess, S. R., Tatham, A. S., Shewry, P. R., Chan, H. W. S., & Morgan, M. R. A. (1990). Characterization of a panel of monoclonal anti-gliadin antibodies. *Journal of Cereal Science, 11*, 89–101.

Payne, P. I., Corfield, K. G., & Blackman, J. A. (1979). Identification of high molecular-weight subunits of glutenin whose presence correlates with bread-making quality in wheats of related pedigree. *Theoretical and Applied Genetics, 55*, 153–159.

Redl, A., Guilbert, S., & Vergnes, B. (1998). Wheat proteins as polymeric materials: rheological properties of plasticized gluten. In *Progress of trends of rheology V, European 5th rheology conference* pp. 61–62.

van Lonkhuijsen, H. J., Hamer, R. J., & Schreuder, C. (1992). Influence of specific gliadins on the breadmaking quality of wheat. *Cereal Chemistry, 69*, 174–177.

Wall, J. S. (1979). In D. L. Laidman & R. G. Wyn-Jones (Eds.), *Recent advances in biochemistry of cereals* (pp. 275–311). London: Academic Press.

Weegels, P. L., Marseille, J. P., Bosveld, P., & Hamer, R. J. (1994). Large-scale separation of gliadins and their bread-making quality. *Journal of Cereal Science, 20*, 253–264.

Xu, J., Bietz, J. A., Felker, F. C, Carriere, C. J., & Wirtz, D. (2001). Rheological properties of vital wheat gluten suspensions. *Cereal Chemistry, 78*(2), 181–185.

Xu, J., Tseng, Y., Carriere, C. J., & Wirtz, D. (2002). Microheterogeneity and microrheology of wheat gliadin suspensions studied by multiple-particle tracking. *Biomacromolecules, 3*(1), 92–99.