

Stress Relaxation Behavior of Wheat Dough, Gluten, and Gluten Protein Fractions

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ABSTRACT

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Relaxation behavior was measured for dough, gluten and gluten protein fractions obtained from the U.K. biscuitmaking flour, Riband, and the U.K. breadmaking flour, Hereward. The relaxation spectrum, in which relaxation times (τ) are related to polymer molecular size, for dough showed a broad molecular size distribution, with two relaxation processes: a major peak at short times and a second peak at times longer than 10 sec, which is thought to correspond to network structure, and which may be attributed to entanglements and physical cross-links of polymers. Relaxation spectra of glutes were similar to those for the corresponding

doughs from both flours. Hereward gluten clearly showed a much more pronounced second peak in relaxation spectrum and higher relaxation modulus than Riband gluten at the same water content. In the gluten protein fractions, gliadin and acetic acid soluble glutenin only showed the first relaxation process, but gel protein clearly showed both the first and second relaxation processes. The results show that the relaxation properties of dough depend on its gluten protein and that gel protein is responsible for the network structure for dough and gluten.

Wheat gluten proteins mainly comprise gliadin and glutenin (Schofield 1986). During dough mixing, gluten proteins are hydrated and form a three-dimensional network, which is responsible for the unique viscoelastic property of dough. Glutenin polymers are largely responsible for variations in breadmaking performance among different cultivars (Orth and Bushuk 1972; Weegels et al 1996; MacRitchie and Lafiandra 1997). Due to the close correlation of high molecular weight (HMW) glutenin polymers with baking performance, much recent research has focused on the measurement of molecular weight distribution (MWD) and structure of the HMW glutenin polymers (Southan and MacRitchie 1999). Gluten proteins comprise a broadly bimodal MWD (Gupta et al 1993; MacRitchie and Lafiandra 1997). Gliadins are single-chain globular proteins with MW ranging from 3×10^4 to 8×10^4 , while glutenins are multiple-chain linear polymeric proteins in which individual polypeptides are thought to be linked by inter-chain disulfide bonds to give a wide MWD range between 10^5 to 10^7 (Wahlund et al 1996; Carceller and Aussenac 2001). Based on knowledge of the structure and MWD of gliadin and glutenin, a network structure of gluten was suggested by Belton (1998) where the linear proteins interact with each other through a loop and train mechanism and disulfide bonding, and linear chains interact with the globular gliadin proteins by nonbonding forces such as Van de Waals interactions. The number of linear-linear protein interactions as well as the number of linear-globular protein interactions will depend on the effective length of linear proteins. Singh and MacRitchie (2001) suggested that there are entanglements between gluten protein polymers, which will contribute the resistance to deformation and slippage rate between polymers and the number of entanglement points depend on the length and MW of protein polymers. The measurement of glutenin polymer molecular weight is a challenging topic in cereal science. As the techniques have been developed, the MWD of gluten proteins has been measured more accurately (Bottomley et al 1982; Bietz 1984; MacRitchie 1984; Southan and MacRitchie 1999; Skerritt et al 1999; Veraverbake et al 1999). However, due to the poor solubility of the HMW glutenin polymers, the measurement of their true molecular weight and structure still remains to be explored.

From polymer science studies, it is widely accepted that molecular weight and structure of polymers are intimately linked

to their rheological behavior and ultimately to their end-use performance. For linear, undiluted monodisperse polymers, below a critical MW, small polymers are relatively mobile and zero-shear viscosity (η_0) increases linearly with increasing MW. At a critical MW, characteristic for each polymer, viscosity starts to increase rapidly with increasing MW, following a relationship $\eta_0 = MW^{3.4}$ (Graessley 1982; Macosko 1994). Above this critical MW, the polymers start to entangle, which gives rise to the observed rapid increase in zero-shear viscosity. According to the modified Rouse theory for concentrated polymer solutions, cross-links and entanglements of polymers play a crucial role in interpreting their viscoelastic properties, in contrast to dilute polymer solutions, where hydrodynamic interactions between molecules are not important (Ferry 1969). At a certain temperature, the relaxation modulus is proportional to moles (ν) of strands between cross-links and entanglements points of polymer with given values of molecules ($G(t) \propto \nu$), and relaxation time is proportional to the number of entanglement loci per molecule ($\tau_p \propto Q_e$) and ξ_0 (molecular friction constant). At high molecular weights, ξ_0 increases to its limiting value ξ_{00} , thus the relaxation time is related to Q_e . Moreover, the concentration of polymers and temperature at which the measurements are made are also important factors to affect the rheological properties of polymers. As Singh and MacRitchie (2001) pointed out, dough is a concentrated and polydisperse polymer system. Rao et al (2001) suggested that stress relaxation measurements would apply to hydrated gluten even though the degree of flexibility of its molecular structure is not well known because the relaxation modulus will reflect both entropic elasticity due to entanglements and enthalpic contributions due to physical cross-links. Furthermore, it was reported that the molecular entanglement of HMW glutenin polymers in dough under large deformation could give rise to strain hardening, which has been shown to be important in determining the strength of dough and stability of gas cells in breadmaking dough during proving and baking expansion (Dobraszczyk and Roberts 1994; Dobraszczyk 1999; Dobraszczyk et al 2003). Therefore, a relatively small variation in the highest end of the MWD and entanglement density can give rise to a large increase in zero-shear viscosity and strain hardening and would be expected to have a critical influence on baking quality.

Rheological tests are increasingly being used to calculate the MWD of monodisperse or polydisperse polymer melts from viscoelastic properties such as relaxation modulus and relaxation time (McGrory and Tuminello 1990; Wasserman 1995; Leonardi et al 2002), and these methods have some obvious advantages over biochemical methods such as size-exclusion high-pressure liquid chromatography (SE-HPLC) and gel-permeation chromatography (GPC).

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graphy (GPC) when they are used for determining the molecular size distribution of very large polymers that are not very soluble. Rapid methods for the characterization of the HMW gluten proteins would be highly desirable for the understanding of the physical mechanisms responsible for variations in baking quality. The relaxation curve and spectrum have been used to identify the structure of gluten (Mita and Bohlin 1983). The stress relaxation behavior of dough and gluten were characterized as two relaxation processes by Bohlin and Carlson (1981), with the first one occurring at short relaxation times (0.1–0.5 sec) and the second one occurring at relaxation times >10 sec. Rao et al (2000) showed that extra-strong doughs exhibited a characteristic bimodal distribution of relaxation times with the second peak clearly discriminating between cultivars with varying strength and quality. The stress relaxation behavior appeared to reflect the expected qualitative differences in the underlying MWD of the gluten proteins, particularly glutenin polymers, with the occurrence of a distinct maximum in the stress relaxation spectrum at longer relaxation times, indicating the presence of a well-developed network structure. Stress relaxation measurements were also carried out for doughs with different strengths (Safari-Ardi 1998), at small strain amplitudes (0.1%), doughs with different strengths showed no difference in their relaxation behavior, but at a range of large strains ($\leq 29\%$), the relaxation behavior was better correlated with the strength of dough. Uthayakumaran et al (2002) investigated the effects of HMW glutenin subunits and molecular size on the rheological properties of doughs, and found that although the rheological parameters G' and G'' decreased with increasing MW and the number of HMW glutenin subunits, relaxation times increased with increasing MW. Thus, relaxation properties of

dough are thought to relate well to MWD and particularly to entanglements of HMW glutenin polymers and may be used as a rapid method of discriminating variations in MWD between cultivars that vary in baking quality. The aim of this study was to characterize the relaxation behavior of gluten protein fractions, as well as the corresponding dough and gluten derived from flours with different baking qualities to get a better understanding of the importance of structure and interaction of these proteins to the strength and quality of dough and gluten.

MATERIALS AND METHODS

Flour Samples

Flour samples were obtained from two the U.K. wheat cultivars (cvs): a moderately strong breadmaking flour, Hereward and a weak biscuitmaking flour, Riband, harvested in 1999 and provided by Heygates Ltd, Bugbrooke, Northampton, U.K. The analytical and baking performance of the flours are given in Table I. The composition of HMW glutenin subunits of flours are listed in Table II.

Preparation of Gluten Samples

Flour (1,000 g) was mixed with a water addition of 59.3% for Hereward and 53% for Riband in a Morton mixer for 60 sec at low speed (120 rpm) and 90 and 30 sec, respectively, at high speed (360 rpm) to achieve optimum mixing for Hereward and Riband. Dough was then removed in a sieve and washed with distilled water for ≈ 20 min by hand until the gluten was obtained. After washing, the gluten was frozen in liquid nitrogen and freeze-dried. The freeze-dried gluten was ground and sieved using a 250- μm sieve. This gluten sample (60 g) was defatted (three times) with

TABLE I
Analytical and Baking Properties of Hereward and Riband Flours

	Hereward	Riband
Protein ^a	10.6% (w/w)	7.5% (w/w)
Water absorption ^b	59.3% (w/w)	53.0% (w/w)
Hagberg Falling No. ^c	403 sec	236 sec
Oven spring ^d	3.2 cm	0.8 cm
Loaf height	15.6 cm	12.6 cm
Loaf volume	1,780 mL	1,450 mL

^a Official Methods of Analysis 992.23. N \times 5.7. (AOAC 1995).

^b Farinograph, Approved Method 54-21 (AACC 2000).

^c Hagberg Falling Number of flour, Approved Method 56-81B (AACC 2000).

^d Breadmaking: Chorleywood Bread Process (CBP) (Axford 1963).

TABLE II
Composition of Glutenin High Molecular Weight Subunits of Hereward and Riband Flours^a

Cultivar	Glu-A1	Glu-B1	Glu-D1
Hereward	Null	7+9	3+12
Riband	Null	6+8	2+12

^a From Khatkar (1996).

TABLE III
Water Content and Protein Content of Gluten and Gluten Protein Fractions

Protein Fraction	Moisture Content ^a (%)	Protein Content ^b (%)
Hglia	0.15	79.60
Hglu	0.12	92.89
Hgel	0.10	72.30
Hgluten	0.09	85.89
Rglia	0.18	85.74
Rglu	0.12	87.00
Rgel	0.10	68.74
Rgluten	0.09	86.89

^a Approved Method 44-19 (AACC 2000).

^b Official Methods of Analysis 992.23. N \times 5.7. (AOAC 1995).

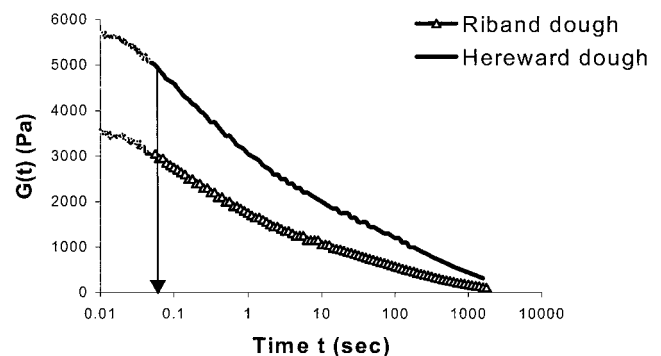


Fig. 1. Stress relaxation curves for Hereward and Riband doughs. Test time at 0.05 sec marked by arrow. Results were means of triplicate separated tests of each sample and standard errors were <10% for test time at 0.1 sec.

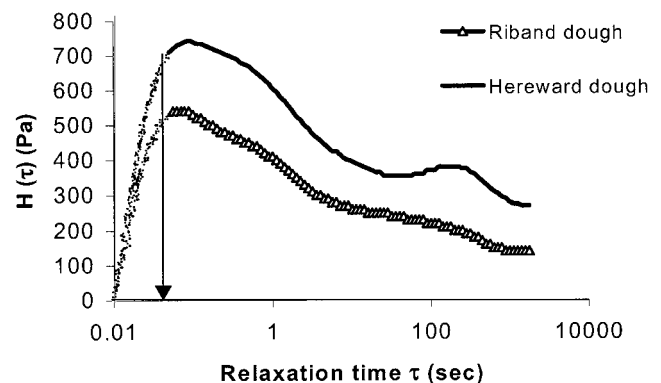


Fig. 2. Stress relaxation spectra for Hereward and Riband doughs. Relaxation time at 0.05 sec marked by arrow. Results were means of triplicate separated tests of each sample and standard errors <10% for test time at 0.1 sec.

400 mL of chloroform under magnetic stirring for 10 min, filtered under vacuum, then dried in an air cupboard overnight.

Preparation of Gluten Protein Fractions

Defatted gluten sample (10 g) was extracted (three times) with 70% (v/v) ethanol (200 mL) at room temperature for 1 hr with magnetic stirring. After centrifuging at $10,000 \times g$ for 30 min, the supernatant was mixed with distilled water, frozen and freeze-dried (gliadin fraction). The residue was washed with 200 mL of distilled water with magnetic stirring at 4°C for 20 min and centrifuged under the same condition as above. The residue was extracted (three times) with (200 mL) 0.05M acetic acid at room temperature for 1 hr with magnetic stirring. After centrifuging at the same conditions as above, the supernatant was frozen and freeze-dried (acetic acid soluble glutenin fraction). The gel layer on the top of residue was scraped off, frozen, and freeze-dried (insoluble gel protein fraction). The water content and protein content of freeze-dried gluten and gluten protein fractions were analyzed and shown in Table III.

Dough samples. Flour (2 g) was mixed with 1.2 mL of distilled water on a 2-g direct drive mixograph (National Mfg., TCMO, Lincoln, NE) to the peak time. All experiments were made at a speed of 88 ± 1 rpm and at room temperature. Dough samples obtained were relaxed for 20 min after mixing in a sealed bottle.

Gluten samples. Defatted gluten (1 g) was hydrated with 2 mL of distilled water by mixing with a spatula and left in a sealed bottle for 20 min.

Gluten protein fractions. The gliadin, acetic acid soluble glutenin, and gel protein were hydrated with 0.6, 1, and 3 mL/g of distilled water, respectively, and the hydrated samples were rested for 2 hr in a sealed bottle. The mixtures of two gluten protein fractions were obtained by mixing gel protein and gliadin or soluble glutenin at 1:1 ratio with 2 mL/g of distilled water with a spatula, then the hydrated protein mixture was rested for 2 hr in a sealed bottle.

Stress Relaxation Test

Stress relaxation test was conducted on a rheometer (VOR, Bohlin Instruments, Cirencester, UK) using parallel-plate geometry (30-mm plate diameter) in shear mode with a 2-mm gap. Stress relaxation behaviors of samples were measured over 1,800 sec at 5% strain with strain rise time 0.1 sec, at 25°C . After the sample was loaded on the rheometer, the excess sample was trimmed off with a razor blade. The dough edges were coated with a thin layer of silicone oil to prevent drying. The sample was rested for another 20 min before the relaxation measurement was taken. The stress relaxation curve was plotted as $G(t)$ versus test time (sec), where $G(t)$ is the relaxation modulus (stress/strain) at any time. The

corresponding relaxation spectrum was calculated from the relaxation modulus by the Bohlin software using Alfrey's rule (Alfrey and Doty 1945):

$$H(\tau) = -(dG(t)/d\ln t)t = \tau$$

where τ is the relaxation time and the value ($H\tau$) in the spectrum represents the intensity of relaxation process at that particular time on a logarithmic scale. The rapid application of strain (5% with a rise time of 0.1 sec) can give rise to inertial effects such as force oscillations that can distort the data and lead to an inaccurate relaxation spectrum at short times. Data below half of the rise time (0.05sec in this case) probably do not represent the true distribution of relaxation times (Rao et al 2001). Therefore, the $G(t)$ and $H(\tau)$ at times <0.05 sec were plotted by a light line and the test time 0.05 sec was indicated by a vertical arrow at 0.05 sec in Figs. 1–8.

RESULTS

Dough and Gluten

The stress relaxation behaviors of dough and gluten obtained from cvs. Hereward and Riband flours were determined for up to 1,800 sec. The stress relaxation curves of two doughs, plotted as relaxation moduli $G(t)$ versus time (sec) are shown in Fig. 1 and the corresponding relaxation spectra are shown in Fig. 2. From the relaxation curves and relaxation spectra for these two doughs, two relaxation processes were distinguished over the whole relaxation time: one occurring at short relaxation times, and the another occurring the longer relaxation times from 10 to 1000 sec. These results are in agreement with the results from Bohlin and Carlson (1981). It could also be seen that $G(t)$ and $H(\tau)$ for the bread-making flour, Hereward, were higher than that of Riband over the whole relaxation time. The relaxation behaviors of the hydrated gluten samples obtained from both doughs were very similar to those for the corresponding doughs. The second peak in relaxation spectrum for Hereward gluten was more pronounced than that for Riband gluten. (Fig. 3). This is good evidence that the relaxation behavior of dough derives from its gluten.

Gluten Protein Fractions

Defatted gluten samples from Hereward and Riband flours were further fractionated into gliadin, acetic acid soluble glutenin, and gel protein fractions. The freeze-dried protein samples of those fractions were hydrated with different amounts of distilled water and then the stress relaxation behaviors were measured.

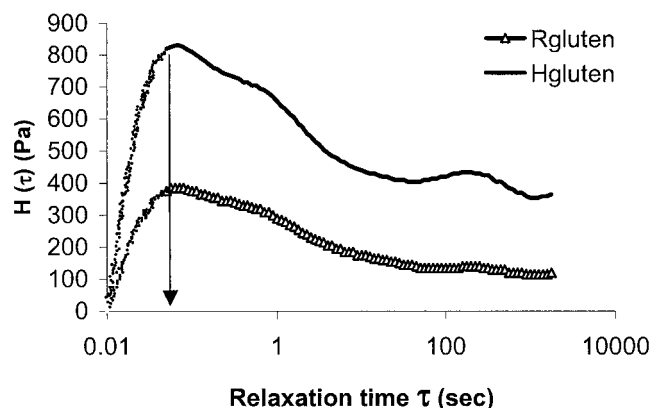


Fig. 3. Stress relaxation spectra for Hereward gluten (Hgluten) and Riband gluten (Rgluten). Relaxation time at 0.05 sec marked by arrow. Results were means of triplicate separated tests of each sample and standard errors $<10\%$ for test time at 0.1 sec.

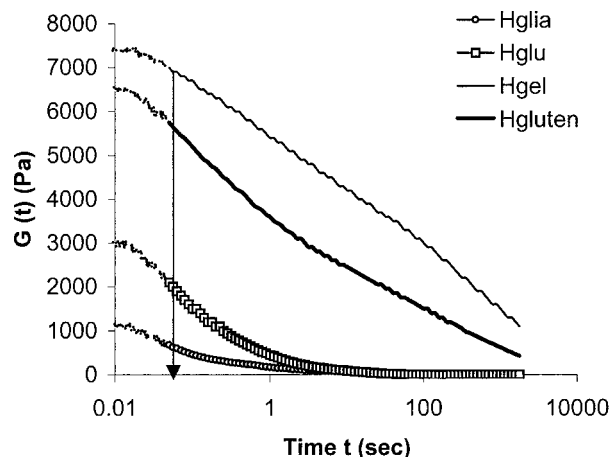


Fig. 4. Stress relaxation curves for Hereward gliadin (Hgliadin), Hereward soluble glutenin (Hglu), Hereward gel protein (Hgel) and Hereward gluten protein (Hgluten). Test time at 0.05 sec marked by arrow. Results were means of triplicate separated tests of each sample and standard errors $<10\%$ for test time at 0.1 sec.

For Hereward gluten protein fractions (Figs. 4 and 5), the hydrated gliadin proteins (0.6 mL/g) had the lowest $G(t)$, $H(\tau)$ and shortest (<10 sec) relaxation time (τ) in three protein fractions. A large peak with a small shoulder was observed at short relaxation times and total relaxation time was shorter than 10 sec. The hydrated soluble glutenin had a much higher $G(t)$ and $H(\tau)$ and a slightly longer relaxation time compared with the hydrated gliadin, its relaxation spectrum also had a large peak over a broader relaxation time and completely relaxed by ≈ 10 sec. Again, only the first relaxation process was observed for this protein fraction.

However, the hydrated gel protein (3 mL/g) showed two relaxation processes occurring in its relaxation spectrum: the first one occurring at short relaxation times, and the second one occurring at relaxation times between 10 and 1,000 sec, which corresponds to the second peak in the relaxation spectrum of Hereward dough and gluten.

The relaxation behavior of protein fractions of Riband was very similar to those of the corresponding fractions of Hereward (Fig. 6). The relaxation spectra for the hydrated gliadin and the soluble glutenin showed the first relaxation process. The hydrated soluble glutenin had a higher value of $G(t)$ and $H(\tau)$ than the hydrated gliadin. The gel protein also showed two relaxation processes occurring at the short relaxation times and the longer relaxation times.

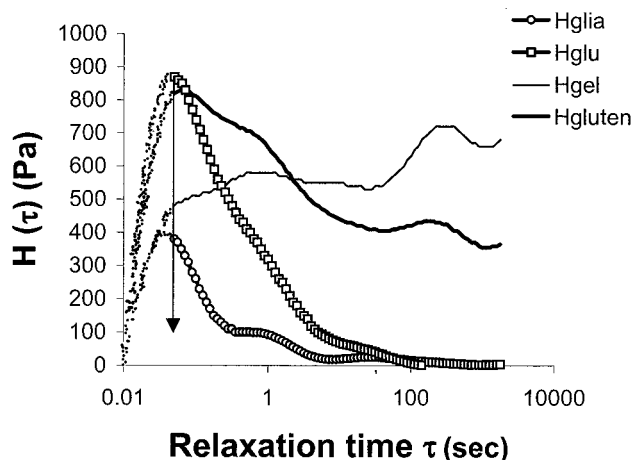


Fig. 5. Stress relaxation spectra for Hereward gliadin (Hgliadin), Hereward soluble glutenin (Hglu), Hereward gel protein (Hgel) and Hereward gluten protein (Hgluten). Relaxation time at 0.05 sec marked by arrow. Results were means of triplicate separated tests of each sample and standard errors $<10\%$ for test time at 0.1 sec.

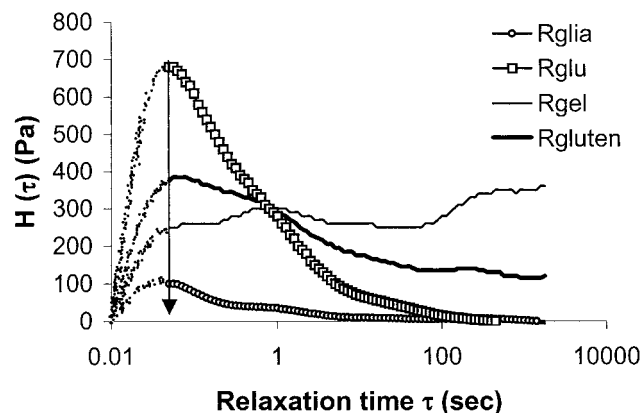


Fig. 6. Stress relaxation spectra for Riband gliadin (Rglia), Riband soluble glutenin (Rglu), Riband gel protein (Rgel) and Riband gluten protein (Gluten). Relaxation time at 0.05 sec marked by arrow. Results were means of triplicate separated tests of each sample and standard errors $<10\%$ for test time at 0.1 sec.

Relaxation Behavior of Protein Fraction Mixtures

After the relaxation behavior of each protein fraction was characterized, the gel protein of Hereward was mixed with its gliadin or acetic acid soluble glutenin at a 1:1 ratio, respectively, and the relaxation behavior of these hydrated mixtures was measured. When the gel protein was mixed with its gliadin fraction and hydrated (2 mL/g), the relaxation behavior of this mixture was significantly different from the relaxation behavior of the gel protein (Fig. 7). There was a large increase in the $H(\tau)$ value in the first peak and a considerable decrease in the $H(\tau)$ value in the second peak compared with that of gel protein. As a result, the second peak in relaxation spectrum for the mixture of gel protein and gliadin almost disappeared. However, when the gel protein was mixed with acetic acid soluble glutenin at a 1:1 ratio and hydrated (2 mL/g), the relaxation behavior was similar to that for the gel protein (Fig. 8). Two relaxation processes clearly occurred in the relaxation spectrum for the mixture of gel protein and soluble glutenin, but the second peak had a lower $H(\tau)$ value and appeared at shorter relaxation times than that for the gel protein.

DISCUSSION

From the results shown above, the relaxation behavior of dough and gluten shows two relaxation processes that occurred at short relaxation times and the longer relaxation times from 10 to 1,000 sec, respectively, which is typical relaxation behavior for polymers

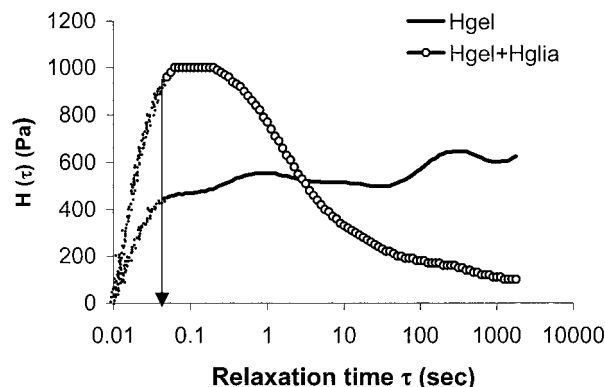


Fig. 7. Stress relaxation spectra for Hereward gel protein (Hgel) and the mixture of gel protein and gliadin at 1:1 ratio (Hgel+glia). Relaxation time at 0.05 sec marked by arrow. Results were means of triplicate separated tests of each sample and standard errors $<10\%$ for test time at 0.1 sec.

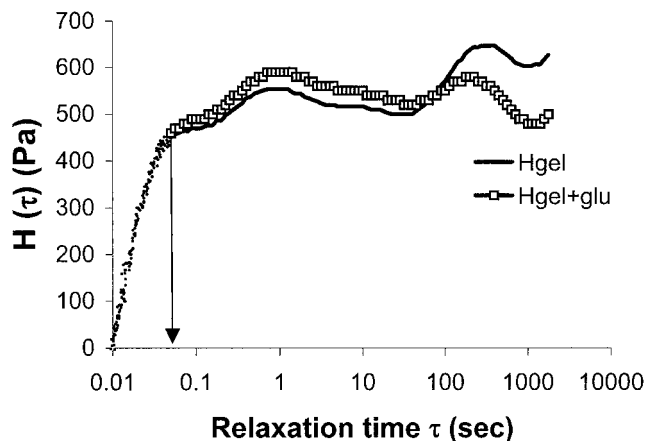


Fig. 8. Stress relaxation spectra for Hereward gel protein (Hgel) and the mixture of gel protein with soluble glutenin at 1:1 ratio (Hgel+glu). Relaxation time 0.05 sec marked by arrow. Results were means of triplicate separated tests of each sample and standard errors $<10\%$ for test time at 0.1 sec.

with a broad molecular size distribution and network structure. These results are in agreement with those from Bohlin and Carlson (1981) and Mita et al (1983). Dough and gluten obtained from strong flour, Hereward, had a higher $G(t)$ and $H(\tau)$ over the whole relaxation time than those from the weak flour, Riband. Furthermore, dough and gluten from Hereward also had a stronger second relaxation process than that of Riband. This indicates that it has a stronger network structure, which could be due to entanglements, physical cross-links, or a combination of both. According to the structural model of gluten protein (Belton 1998), the network structure can be envisaged as interactions between linear glutenins and linear-globular glutenin and gliadin and suggested to depend on entanglements between protein polymers (Singh and MacRitchie 2001).

The characterization of relaxation behavior of gluten protein fractions, i.e. gliadin, acetic acid soluble glutenin, and gel protein has given insight into the molecular process governing the relaxation behavior of dough and gluten and provides basic concepts that can be used to understand the behavior of this complex polydisperse system. Gliadins, soluble in 70% ethanol are mainly globular proteins with molecular weights ranging from 30,000 to 80,000, contaminated with a small amount of glutenin when they are extracted (Shewry et al 1983; Shewry and Tatham 1997). A large peak with a small shoulder occurring at short times in relaxation spectrum for gliadin may represent two components: one (major peak) is related to the monomeric gliadin and the other (small shoulder) is for the co-extracted glutenin proteins. No network structure, which has a long relaxation time, was observed. Acetic acid soluble glutenin has a broad range of molecular weight distribution from 10^5 to 10^7 (Wahlund et al 1996; Skerritt et al 1999; Carceller and Aussenac 2001). The results in the present study show that its relaxation spectrum had only one relaxation process with a broader relaxation time and higher $G(t)$ and $H(\tau)$ compared with that for gliadin. This probably means that the molecular size of soluble glutenin is larger than that of gliadin. A higher $G(t)$ observed for soluble glutenin hydrated at a higher water absorption than that for gliadin hydrated at a lower water absorption indicates that the interaction between soluble glutenin proteins is stronger than that between gliadin proteins, but no network structure that relaxed at a long time scale was observed in the soluble glutenin protein gel.

Insoluble gel proteins are proposed to have a very large molecular weight (Graveland et al 1985). The relaxation spectrum of gel protein showed two relaxation processes. This demonstrated a strong network structure in gel proteins, which is responsible for the occurrence of the second peak in relaxation spectrum for dough and gluten. It is suggested that this network structure, which relaxed at a long relaxation time, is attributed to entanglements of protein polymers (Singh and MacRitchie 2001) or the interactions of linear proteins through the loop and train mechanism (Belton 1998).

According to Wasserman (1995) and McGrory (1990), the relaxation time can be used to calculate the molecular weight of polymers for monodisperse and polydisperse polymer melts. Due to the poor solubility of the gel proteins, it is not possible to measure their molecular size distribution by biochemical methods. The results in the present study provide a strong evidence that gel proteins have a much larger molecular size than gliadin and soluble glutenin, and have a strong network structure. Furthermore, the more pronounced peak in relaxation spectrum for the gel protein from Hereward flour showed a stronger network than that for Riband. This difference may account for the variation in baking performance between the two flours.

Although the relaxation behavior of gliadin was similar to that of soluble glutenin, it was surprising to find that they were able to change the relaxation behavior of gel proteins in different ways when they were mixed with gel proteins. The gliadin fraction had a very strong ability to enhance the first relaxation process and to

weaken the second relaxation process. Acetic acid soluble glutenin only slightly decreased the $H(\tau)$ value and the relaxation time for the second relaxation process. For a polydisperse polymer system, Termonia and Smith (1992) found two parameters were useful in characterizing polydisperse linear polymer systems for interpreting their rheological behavior, which are the molecular size and polymer component ratio. The increase in the value of first peak in the relaxation spectrum represents an increase of contribution of the polymers with small molecular size, and vice versa, the decrease of value of the relaxation spectrum at longer relaxation times (>10 sec) implies a smaller contribution of polymers with big molecular size. However, at the same ratio, gliadin dominated the relaxation behavior of the mixture with gel protein, but the relaxation behavior of the mixture of soluble glutenin with gel protein was still similar to that for gel protein. This result suggests that the relaxation behavior of a polydisperse protein mixture may not be simply additive and the interaction of protein molecules may play an important role in the rheological behavior of protein mixtures.

Even though the mixture of Riband gluten proteins was not measured, it is very likely that Riband gliadin and soluble glutenin have the similar effects on the stress relaxation behaviors of the protein mixture with gel protein when they are mixed, because those corresponding gluten protein fractions had such similar relaxation behaviors to that from Hereward flour (Figs. 5 and 6).

When rheological tests are made with dough, relatively high standard errors ($> 5\%$) are common. In the present study, the method for preparing dough and gluten samples was very important for achieving reproducible results. The dough had to be mixed until fully developed (to mixing time) on the mixograph. The gluten sample needed to be ground to similar particle size and to be fully hydrated before testing. Furthermore, the water content of samples and the temperature at which the measurement was made should be constant throughout the test. Even though standard errors at the test time 0.1 sec were $<10\%$, the means of relaxation moduli for dough and gluten for two cultivars and for different protein fractions were significantly different ($P > 0.05$ level). Therefore, the tests were relatively sensible and the results were reproducible.

CONCLUSIONS

The relaxation behavior of dough was similar to that of its gluten, for both strong and weak flours, and had two distinct relaxation processes. The dough and gluten of strong flour had a higher relaxation modulus $G(t)$ and relaxation intensity $H(\tau)$ over the whole relaxation time than those from weak flour.

In gluten protein fractions, gliadin and soluble glutenin had one relaxation process, indicating no network structure occurred in those protein gels, but gel protein had two relaxation processes, which corresponded to those for the dough and gluten. The second relaxation process showed network structure, which may be attributed to the protein molecular entanglements and physical cross-links.

Gliadin and acetic acid soluble glutenin had different effects on the relaxation behavior of the gel protein. Gliadin enhanced the first relaxation process and diminished the second one, but soluble glutenin only slightly reduced the relaxation time for second process.

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