

# Synergistic and Additive Effects of Three High Molecular Weight Glutenin Subunit Loci. I. Effects on Wheat Dough Rheology

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## ABSTRACT

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The high molecular weight glutenin subunits (HMW-GS) play an important role in governing the functional properties of wheat dough. To understand the role of HMW-GS in defining the basic and applied rheological parameters and end-use quality of wheat dough, it is essential to conduct a systematic study where the effect of different HMW-GS are determined. This study focuses on the effect of HMW-GS on basic rheological properties. Eight wheat lines derived from cvs. Olympic and Gabo were used in this study. One line contained HMW-GS coded by all three loci, three lines were each null at one of the loci, three lines were null at two of the loci and one line null at all three loci. The flour protein

level of all samples was adjusted to a constant 9% by adding starch. In another set of experiments, in addition to the flour protein content being held at 9%, the glutenin-to-gliadin ratio was maintained at 0.62 by adding gliadin. Rheological properties such as elongational, dynamic, and shear viscometric properties were determined. The presence of *Glu-D1* subunits (5+10) made a significantly larger contribution to dough properties than those encoded by *Glu-B1* (17+18), while subunit 1, encoded by *Glu-A1*, made the least contribution to functionality. Results also confirmed that HMW-GS contributed to strength and stability of dough.

Understanding the role of the various constituents of wheat flour in determining the functional properties of dough has been a major endeavor throughout the 20th century. With this knowledge, better wheat cultivars can be bred and more economical manufacturing technologies can be developed. The protein in wheat is mainly responsible for inherent differences in baking quality (Finney 1943), with both quantity and quality of the protein being important (Finney and Barmore 1948). It has been well established that two classes of proteins, glutenins and gliadins, are the key components in determining the rheological properties of dough as they interact to form the gluten matrix during dough mixing. Glutenins are polymers of high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS), which differ in their mobility during SDS-PAGE. The HMW-GS have the largest role in defining dough strength (MacRitchie 1992) and possibly stability (Payne 1987).

Bread wheat (*Triticum aestivum* L. emend Thell.) is a hexaploid species with three genomes designated A, B, and D. The *Glu-1* loci on the long arms of the homoeologous Group 1 chromosomes code for the HMW-GS, and are designated *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively. Each locus generally codes for two subunits, “x” and “y”, although the *Glu-A1* y-type gene is usually not expressed. Each locus also has several alleles, differences between which account for a considerable proportion of the variation in dough strength (Payne et al 1987). Null alleles, where no subunits are produced at all, have been developed at each of the three *Glu-1* loci (Lawrence et al 1988). This set of lines provides a valuable resource for identifying the effect of each *Glu-1* locus on overall rheology and end-product quality. The set has already been used for studies on polymeric protein formation (Gao and Bushuk 1993; Gupta et al 1995), but without correction to a constant protein content.

For these reasons, basic rheological tests (elongational, shear viscometry, and dynamic oscillatory tests) and end-use quality tests (breadmaking) were conducted. Fundamental or basic rheological

tests are considered to be valuable tools in determining dough functionality and unlike the empirical dough measurement tests (mixograph, farinograph, extensigraph, and alveograph), are founded on parameters of basic physics (Levine 1987). The rheological properties of dough change considerably during every baking phase and the viscoelastic properties of dough have a profound effect on the finished product (Walker and Hazelton 1996). The basic rheological measurements can be used for constitutive modeling of bread dough and this may lead to predictions of dough processing behavior.

## MATERIALS AND METHODS

The eight flour samples differed by the activity (designated +) or inactivity (designated – or null) of each of the three glutenin loci (Table I). The ash content was determined using Approved Method 08-01 (AACC 2000). The nitrogen content of the flours was determined by the Dumas total combustion method using an elemental analyzer (CHN-1000, Leco Inc., St Joseph, MI). The protein content (%) was determined as  $N \times 5.7$ . The moisture content of the flours was determined by a near infrared spectrometer with sample transport (Foss NIRSystems 6500, Silver Spring, MD). Flour protein level of all samples was adjusted to a constant 9% by adding commercial wheat starch (1.6% protein). The ratio of glutenin to gliadin, ratio of high molecular weight glutenin subunits to low molecular weight glutenin subunits (HMW-GS/LMW-GS ratio) and the unextractable polymeric protein percentage (% UPP) (Table I) of the samples were determined using reversed-phase HPLC (Marchylo et al 1989) and size-exclusion HPLC (Singh et al 1990; Batey et al 1991; Gupta et al 1993), respectively.

The effect of maintaining the glutenin-to-gliadin ratio constant was investigated in a subset of the flours. The choice of materials was limited to four by the quantity of flour available. Gliadin (85% protein) was extracted from flour of the line null for all three HMW-GS loci using the mild pH extraction method of MacRitchie (1985). Lyophilized gliadin was added to + + +, + – + and – – + flours to achieve a uniform glutenin-to-gliadin ratio of 0.62, as found in the – – – sample. Commercial starch was also added to maintain the constant protein content of 9%.

Mixing was conducted at a water absorption of 57%. Blended flour and water were mixed in a 10-g mixograph to peak dough development and rheological measurements were then made. Mixing was done in triplicate for each sample and the mean peak dough development time was calculated from the mixing curve (Gras et al 1990).

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**TABLE II**  
Summary Analysis of Variance (mean square values) of Fundamental Rheology Parameters in a Set of Eight Lines of Wheat Differing in High Molecular Weight Glutenin Subunits (HMW-GS)<sup>a</sup>

Source of Variation	df	%UPP	HMW-GS to LMW-GS Ratio	Max. Shear	Viscosity			Rupture Strain	G'	G''	tan δ	Stress Relaxation (1 sec)	
					Log Shear	Max. Elong.	Log Elong.					Small Strain	Large Strain
<i>Glu-A1</i>	1	233.6***	0.009158***	57.08***	0.8904***	4519***	5.500***	0.1388***	10.07***	0.9212***	0.0470 <sup>ns</sup>	1.118 <sup>ns</sup>	1.199 <sup>ns</sup>
<i>Glu-B1</i>	1	810.3***	0.1178***	70.48***	0.7310***	5065***	6.097***	0.1502***	32.92***	0.9014***	21.87***	229.9***	21.07***
<i>Glu-D1</i>	1	1.173***	0.05780***	137.8***	1.804***	10412***	10.03***	0.02481**	4.400***	2.978***	17.64***	13.16**	2.295**
<i>Glu-A1</i> × <i>Glu-B1</i>	1	18.58***	0.000624 <sup>ns</sup>	1.188*	0.000008 <sup>ns</sup>	8.006**	0.7331***	0.1661***	5.190***	0.4602***	0.0018 <sup>ns</sup>	6.288*	1.677*
<i>Glu-A1</i> × <i>Glu-D1</i>	1	48.22***	0.000011 <sup>ns</sup>	1.113*	0.1418***	643.8***	0.3929***	0.2576***	1.359**	0.4920***	2.449**	20.41***	2.496*
<i>Glu-B1</i> × <i>Glu-D1</i>	1	49.54***	0.01434***	10.27***	0.01380*	709.9***	0.4622***	0.02976**	0.0522 <sup>ns</sup>	0.0547*	1.549**	0.676 <sup>ns</sup>	5.221**
<i>Glu-A1</i> × <i>Glu-B1</i> × <i>Glu-D1</i>	1	0.922 <sup>ns</sup>	0.000027 <sup>ns</sup>	2.856***	0.03456**	207.8***	0.000026 <sup>ns</sup>	0.2627***	1.521**	0.4666***	1.656**	5.676*	3.312**
Residual	8	0.7043	0.0002634	0.1076	0.001487	0.6637	0.003462	0.001744	0.0547	0.009391	0.1238	0.6376	0.2333

<sup>a</sup> \*, \*\*, \*\*\* = significant at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ , respectively; ns = not significant.

**TABLE III**  
Means of Fundamental Rheology Parameters in a set of Eight Lines of Wheat Differing in High Molecular Weight Glutenin Subunits (HMW-GS)

Activity <sup>a</sup>	Shear Viscosity		Elongational Viscosity		Rupture Strain	G'	G''	tan δ	Stress Relaxation (1 sec Pa)	
	(kPa.sec)	(log)	(kPa.sec)	(log)					Small Strain	Large Strain
+++	16.9	9.73	115.1	11.6	2.58	9.6	3.47	19.8	5.7	3.4
++	13.9	9.54	74.6	11.2	2.59	10.1	3.63	19.9	6.4	3.7
+-	11.4	9.34	72.0	11.2	2.42	12.4	4.08	18.0	15.3	8.4
+	10.8	9.28	45.3	10.7	2.58	10.8	4.27	21.6	6.0	5.5
-+	7.8	8.96	19.9	9.9	2.54	15.1	4.73	17.4	11.1	5.5
-	5.1	8.53	15.8	9.7	2.60	11.3	4.41	21.3	8.9	5.4
---	6.8	8.82	14.4	9.6	2.76	13.2	4.90	20.4	14.1	6.4
SE <sup>b</sup>	0.58	0.027	0.23	0.042	0.030	0.16	0.069	0.25	0.56	0.34

<sup>a</sup> Activity (designated +) or inactivity (designated - or null).

<sup>b</sup> Standard error.

**TABLE IV**  
Effects of Maintaining a Constant Glutenin-to-Gliadin Ratio of 0.62 on Elongational and Shear Viscosity

Activity <sup>a</sup>	Elongational Viscosity		Shear Viscosity	
	(kPa.sec)	(log)	(kPa.sec)	(log)
+++	55.0	10.92	12.0	9.43
++	28.0	9.99	11.0	9.31
+-	15.0	9.37	7.0	8.87
+	7.4	8.92	6.2	8.69
SE <sup>b</sup>	0.80	0.041	0.13	0.016

<sup>a</sup> Activity (designated +) or inactivity (designated - or null).

<sup>b</sup> Standard error.

**TABLE V**  
Coefficients of Relaxation Spectra (log H vs. log time)

Activity <sup>a</sup>	Intercept	Slope
+++	3.14	-0.207
++	3.11	-0.256
+-	3.19	-0.249
+	3.24	-0.210
-+	3.24	-0.249
-	3.31	-0.215
---	3.27	-0.238
Standard error	0.0001	0.0001

<sup>a</sup> Activity (designated +) or inactivity (designated - or null).

## Stress Relaxation

The stress relaxation test was carried out on a controlled strain rheometer (Bohlin VOR). Dough was mixed to optimum development time and a 4-g dough piece was mounted in the parallel plate configuration (30 mm diameter, 2 mm gap). Sandpaper, petroleum jelly, and resting time were used as described above. An instantaneous strain (0.1 or 10%) was applied to the sample and the relaxation of the stress was measured. A 40 g.cm torsion bar was used for the low strain and a 315 g.cm torsion bar was used for the high strain. The tests were performed at 25°C. In this test, the top plate was rotated through a small angle and the resulting stress was measured as a function of time. Data is presented as the relaxation modulus  $G = \text{stress/strain}$ . Values from the 1-sec evaluation were extracted for statistical analysis. Because of the overlap of the curves, only four of the eight sets of data are shown in the graphs.

## Relaxation Time Spectrum

The oscillatory shear data (data obtained from frequency sweep tests) were further processed. Relaxation time spectra (Log H v log time) were obtained by mathematical inversion from dynamic data using the nonlinear regularization technique of Honerkamp and Weese (1993). Further details about the mathematical inversion are contained in Friedrich et al (1995) and Phan-Thien and Safari-

Ardi (1998). The region of validity of the spectrum was set by the frequency range of the dynamic data (Phan-Thien and Safari-Ardi 1998). The inversion program computed an estimated error for the spectrum. The region of minimum error approximated the region of validity of the spectrum. The relaxation time spectrum is presented as points on a curve of log H against log relaxation time in seconds.

## Statistical Analysis

Statistical analyses were made using MSUSTAT v 4.1 (Richard E. Lund, Montana State University, Bozeman, MT) and SuperAnova v 1.11 (Abacus Concepts, Berkeley, CA). The eight genotypes were handled first as a single source of variation and second as a set of three factorial sources, where the interaction terms tested for nonadditivity of their effects. Correlations were calculated among the eight means of the parameters measured.

## RESULTS

### Large Strain

Under uniaxial elongation, the elongational viscosity of the dough increased with extension, more rapidly at the highest strain levels (Fig. 1). This sharp increase in elongational viscosity with increasing strain is known as strain hardening. A maximum viscosity value

was reached during this strain-hardening stage, at which point the dough sample ruptured between the plates.

Maximum shear viscosity and maximum elongational viscosity were dependent on the main effects of the individual genes, by the pairwise interactions between them, and by the three-way interaction as well (Table II). The individual genes were responsible for more of the variance than the interactions, with *Glu-D1* having the greatest effect. Because of the broad distribution of the data, covering two orders of magnitude in the case of elongational viscosity, the analysis of the log-transformed data was also inspected, but this showed only two changes: the *Glu-A1* × *Glu-B1* interaction for shear viscosity and the *Glu-A1* × *Glu-B1* × *Glu-D1* interaction for elongational viscosity were no longer significant.

In both elongational and shear viscosity, the pairwise combinations of active genes (single-null genotypes) had higher values than expected from the single active genes (double-null genotypes), and the three-way combination was greater again. The null *Glu-D1* line showed much lower viscosity values than the other single-null lines (Table III). The three lines null at two loci had very low shear and elongational viscosity. The triple-null line showed no strain hardening properties (Fig. 1) as well as the lowest shear and elongational viscosity values (Table III).

Rupture strain also showed a complete range of significant main effects and interactions. In this case, however, the *Glu-D1* HMW-GS had the least effect and the interactions were of comparable size to the main effects, showing that synergy was as important as additivity. The triple-null genotype had much lower values than the other genotypes. Of the remaining samples, the + - + genotype had a lower value than the rest and + - - had a higher value, with the remaining genotypes not significantly different from each other. These results indicate that HMW-GS play an important role in strain hardening properties, with *Glu-D1* having the strongest effect.

Maximum shear and elongational viscosity were very strongly correlated with each other ( $r = 0.971$ ,  $P < 0.001$ ), but neither was significantly correlated with rupture strain.

When the glutenin-to-gliadin ratio was maintained at 0.62, the range of values in elongational and shear viscosity was reduced (Table IV). Both measures of viscosity increased as more HMW-GS were present. Nevertheless, because of the structure of this part of the experiment, it was not possible to test the data for additivity and synergy.

### Protein Size

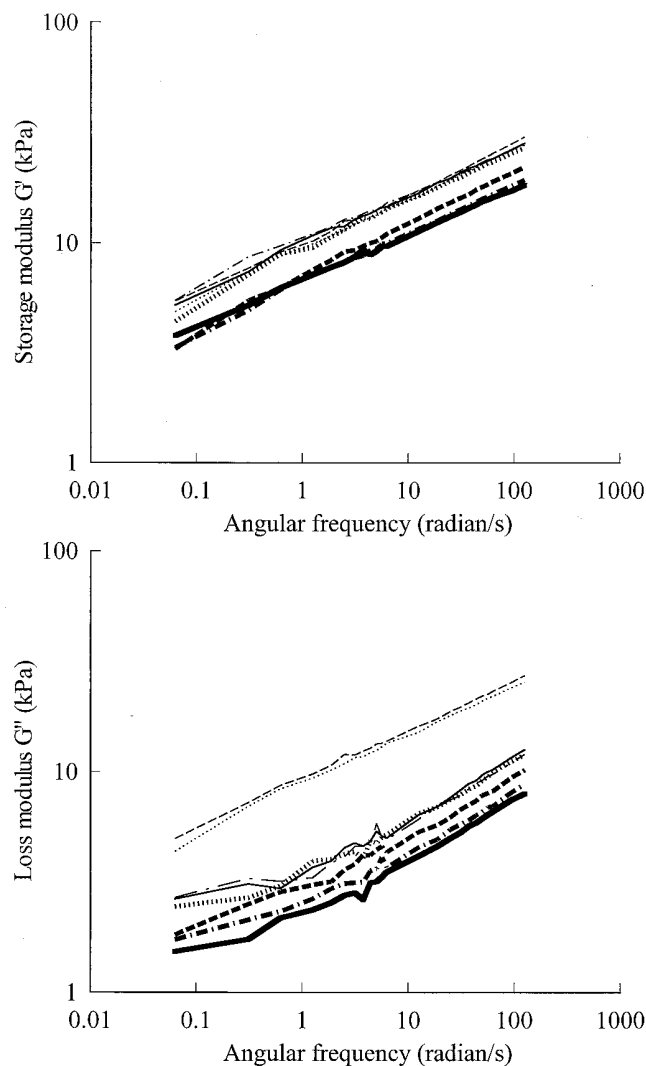
Variation in percent unextractable polymeric protein (% UPP), a measure of molecular size, showed the same pattern of statistical significance as elongational and shear viscosity, except that the three-way interaction was not significant (Table II). The triple-null genotype had the lowest % UPP and the + + + genotype had the highest (Table I). The single-null genotypes (except + + -) had higher values than expected from the double-null genotypes.

The % UPP was very strongly correlated with maximum shear viscosity ( $r = 0.950$ ,  $P < 0.001$ ) and maximum elongational viscosity ( $r = 0.975$ ,  $P < 0.001$ ). The corresponding correlations of shear and elongational viscosity with the HMW-GS to LMW-GS ratio were 0.915 and 0.894 ( $P < 0.01$  for both) and with the glutenin-to-gliadin ratio were 0.822 ( $P < 0.05$ ) and 0.881 ( $P < 0.01$ ).

### Dynamic Tests

Strain sweeps conducted at 1 Hz revealed the linear viscoelastic region of all flours to be below a strain of  $1 \times 10^{-3}$  (Fig. 2). Frequency sweep experiments were therefore performed at strain of  $5 \times 10^{-4}$  within the linear region of the dough.

The subunits encoded at the *Glu-B1* locus had the greatest effect on  $G'$  in the analysis of variance, followed by the *Glu-A1* subunits, the *Glu-A1* × *Glu-B1* interaction, and then the *Glu-D1* subunits (Table II). The *Glu-A1* × *Glu-B1* × *Glu-D1* interaction was significant. The  $G''$ , in contrast, was most strongly affected by the *Glu-D1* subunits. Phase angle showed strong effects of



**Fig. 3.** Effects of high molecular weight glutenin subunit composition on the storage modulus ( $G'$ ) and loss modulus ( $G''$ ) of wheat dough: + + +, — thick; + + -, — thick; + - +, ..... thick; - + +, — · — · thick; + - -, — · — · thin; - + -, ..... thin; - - +, — — thin; - - -, — thin.

subunits controlled by *Glu-B1* and *Glu-D1*, but the effect of the *Glu-A1* was nonsignificant, as was the *Glu-A1* × *Glu-B1* interaction.

Both  $G'$  and  $G''$  were relatively high in the - - + and - - - genotypes (Table III). Both measures showed a general trend of decreasing as the number of glutenin subunits increased (Fig. 3), commensurate with increases in dough strength by other measures.

### Relaxation Time Spectrum

The data for each flour conformed very closely to a straight line of fit when  $\log H$  (relaxation time spectrum) was plotted against the log of time (Fig. 4). The lines crossed and the intercepts and slopes were significantly different (Table V).

### Relaxation Modulus

Between 0.1 and 100 sec, the relaxation modulus data formed a smooth curve (Fig. 5). The actuation time of the rheometer was about 0.05 sec, so data at lesser time scales was unreliable and had to be excluded. Separation of the genotypes was clearer with small strain than with large strain and, in both cases, the + + + genotype was at the bottom, followed by the single null, then the double null, and on the top, the triple null. The plot of log relaxation modulus versus log time was strongly linear ( $r^2 > 0.984$ ) in the range of 0.1–10 sec (small strain) and 0.1–100 sec (large strain). The analysis of variance showed that the contribution of *Glu-B1* to stress

relaxation exceeded that of *Glu-D1*, and that of *Glu-A1* was nonsignificant (Table II). The *Glu-A1* × *Glu-B1* × *Glu-D1* interaction was significant in both cases, showing that stress relaxation was affected in a nonadditive way by the different genes. This was particularly demonstrated by the very high values found in the + + + genotype, which were below only the - - - genotype in the small strain test and were higher than all others in the large strain test (Table III).

### DISCUSSION

The HMW-GS interacted to a significant extent in determining many of the fundamental rheological properties of the doughs, with the whole being more than the sum of the parts. Nevertheless, the main effects of the subunits were generally much larger than their interactions, with *Glu-D1* having the greatest effect on shear viscosity, elongational viscosity, and loss modulus, while *Glu-B1* had the greatest effect on storage modulus, phase angle, and stress relaxation. Part of the synergistic action may have been attributable to changes in the glutenin-to-gliadin ratio as increasing numbers of HMW-GS were present.

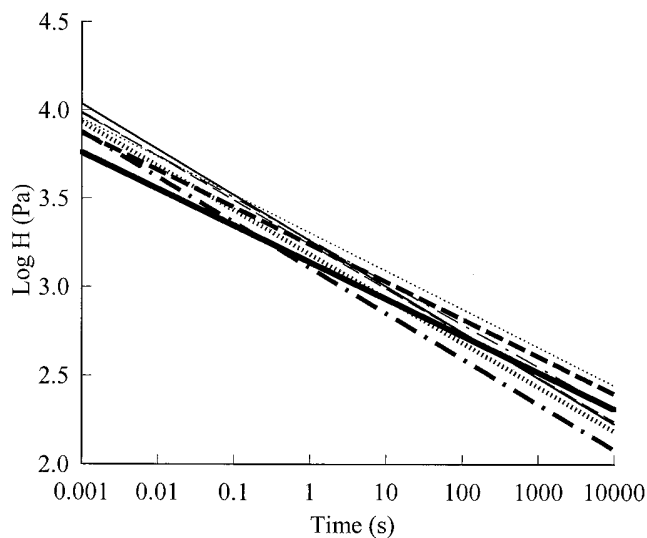
Elongational flow is presumed to be the predominant type of flow occurring in the dough surrounding the inflating gas bubbles during fermentation and baking (van Vliet et al 1992, 1993). Elongation at low strain rate is similar to bread dough fermentation and oven-rise, where the dough surrounding the expanding gas bubbles is extended along the two axes of the bubble surface, while the bubble wall becomes progressively thinner. Strain hardening, the rapid increase in elongational viscosity at higher strain levels, is thought to be responsible for the ability of dough to expand and retain the gas evolved during fermentation and baking. Flours with good baking quality tended to have much greater strain hardening behavior than flours that performed poorly in baking (van Vliet et al 1992, 1993). It can be inferred that the greater strain hardening properties of dough due to HMW-GS indicate that these proteins contribute to good baking quality. The greater effect of the *Glu-D1* subunits on strain hardening is in line with expectations from the substantial effect of the presence of subunits 5 + 10 on breadmaking quality (Payne et al 1987).

Increasing either the concentration of a polymer solution or the polymer size increases the shear viscosity (Vinogradov and Malkin 1980). This increase was observed when the number of HMW-GS was increased, which increased the % UPP of the sample. Thus, in

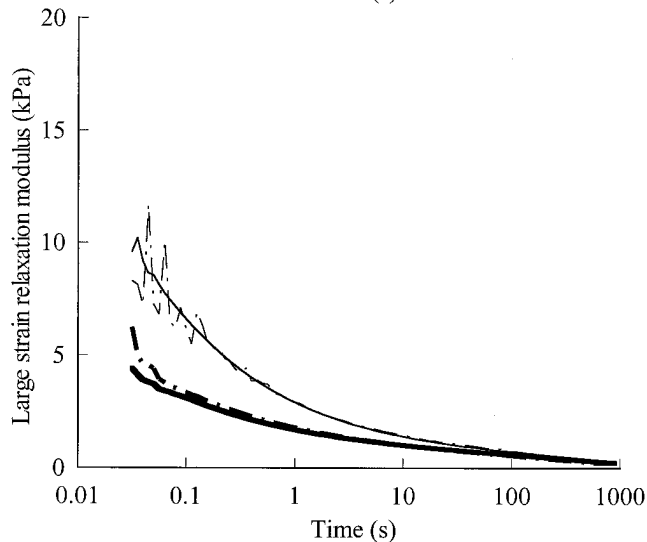
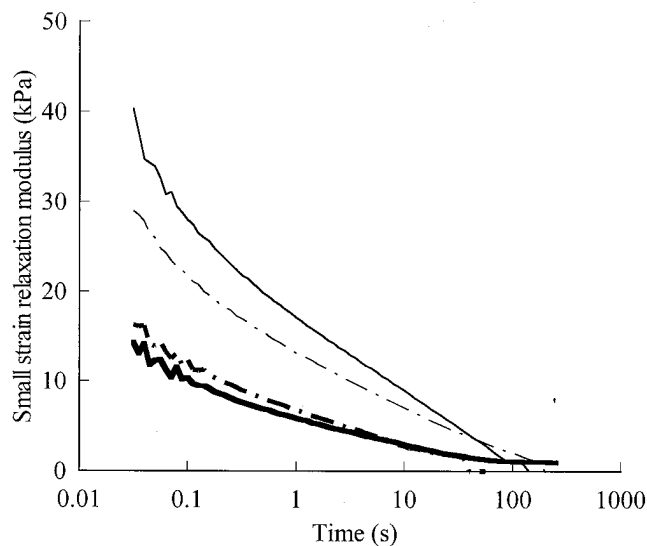
this set of materials, dough functioned at least in part as a polymer solution.

The quality of wheat for breadmaking has been related to the molecular size distribution of the gluten-forming proteins of the flour (MacRitchie 1992; MacRitchie and Lafiandra 1997). The overall size distribution of polymeric protein of flours analyzed using SE-HPLC is one of the most important function-related chemical characteristics in relation to dough properties. The results indicated that dough strength increased with increases in the size distribution of the polymeric proteins. It can be concluded that ultimately it is the size distribution that plays the role in determining dough strength measured as high elongational and shear viscosity.

To get useful information, the dynamic oscillatory measurements needed to be made in the linear viscoelastic region, so that the storage and loss moduli would be independent of the applied stress or strain. In the present work, this end was achieved with a strain of  $5 \times 10^{-4}$ . The linear viscoelastic behavior of wheat dough has been investigated by several authors (Hibberd and Wallace 1966; Smith et al 1970; Navickis et al 1982; Phan-Thien and Safari-Ardi 1998), and various results have been obtained. Though there is no agreement on an exact strain value at which the linear viscoelastic behavior ceases to exist, the results from these studies show that for wheat flour doughs it is <0.2.



**Fig. 4.** Effects of high molecular weight glutenin subunit composition on the relaxation spectra of wheat dough: + + +, — thick; + + -, — thick; + - +, ..... thick; - + +, - · - · thick; + - -, - · - · thin; - + -, ..... thin; - + +, — thin; - - -, — thin.



**Fig. 5.** Effects of high molecular weight glutenin subunit composition on the relaxation modulus of wheat doughs under small strain (0.1 %) and large strain (10 %): + + +, — thick; - + +, - · - · thick; + - -, - · - · thin; - + -, ..... thin; - - -, — thin.

In the present work, both  $G'$  and  $G''$  decreased as dough strength increased. Similar effects have been observed in dynamic viscosity measurements, where a strong baker's flour had a lower viscosity than a weak biscuit flour at high strains (Safari-Ardi and Phan-Thien 1998). There was no significant difference observed in the dynamic viscosity, and no consistent trend in  $\tan \delta$ , which is subject to large experimental errors, making it difficult to discern any trends.

Stress relaxation and  $G'$  were particularly affected by the *Glu-B1* subunits. This is an unexpected result as there is little evidence for a similar bias toward the *Glu-B1* HMW subunits in other quality-related traits.

Relaxation time spectra ( $\log H$  vs  $\log t$ ) were linear, just as are spectra for filled (containing dispersed particles) polymers (Ferry 1961). When allowance is made for the inherent errors in the inversion process and the region of validity of the spectral data ( $0.002 < t < 16$  sec), some conclusions can be drawn from the current data. At the short relaxation time region of the spectrum, the lines with one or more nulls had a tendency to exhibit more of the short relaxation times than the lines with no nulls. At the long relaxation time region of the spectrum, there was some overlap of the data, but generally the lines with few or no nulls had a tendency to exhibit more of the long relaxation times. This is consistent with the null lines having shorter relaxation times (and hence shorter polymer chains) while those without nulls having the longer relaxation times (and hence longer polymer chains). Published relaxation time spectra for bread dough are generally consistent with those shown here, with only one known exception. Recent work by Rao et al (2000) showed relaxation time spectra had a bimodal distribution, but they generated their spectra using Alfrey's rule, an approximate method known to be inaccurate (Ferry 1961). On the other hand, our work and other published literature (Safari-Ardi and Phan-Thien 1998; Phan-Thien and Safari-Ardi 1998) have used exact methods such as mathematical inversion and these are believed to be more reliable (Honerkamp and Weese 1993). Later results of Rao et al (2001) differ from their earlier work and are similar to those presented here.

Relaxation modulus is a measure of the relaxation of material after a sudden strain and is helpful in distinguishing dough types (Safari-Ardi and Phan-Thien 1998). Safari-Ardi et al (1997) have shown that high-strain stress relaxation measurements on certain flour types can bring out subtle differences in doughs. The current data agree closely with spectra of Phan-Thien et al (1997) and Safari-Ardi and Phan-Thien (1998). Mead (1994) has shown that stress relaxation is useful for characterizing the linear viscoelastic properties of polymers in the plateau and terminal region, where information on molecular weight is contained, and also showed that largest stress relaxation times are associated with large molecules. Furthermore, glutenin molecular size distribution plays an important role in dough quality (MacRitchie 1992, Uthayakumaran et al 1999). For example, loaf height is directly proportional to average glutenin molecular weight. Stress relaxation tests may therefore be used to screen samples for an important end-use property. In the current study, the + + + dough had low stress modulus values at short relaxation times compared to the - - - dough. At longer relaxation times, however, the curves merged. Similar trends have been obtained with starch-gluten mixtures and the comparison of gluten doughs with flour doughs (Uthayakumaran, unpublished). We can conclude that the stronger doughs have low relaxation modulus at the short relaxation times. Previous studies by Safari-Ardi and Phan-Thien (1998) indicated that high strain rather than low strain rheology was needed to distinguish between functionally different flours. In this study, however, we were able to distinguish between samples even at very low strains (strain within the linear viscoelastic region).

Increased glutenin-to-gliadin ratio contributes to higher elongational viscosity and shear viscosity (Uthayakumaran et al 2000), so these results raise the question as to whether the effects observed

were related to the HMW-GS or to the contribution of glutenin-to-gliadin ratio. The samples in which both protein content and glutenin-to-gliadin ratio were kept constant confirmed that the presence of all the HMW-GS contributed to the highest elongational viscosity. The differences between the samples were not as pronounced as when only the protein content was kept constant. Shear viscometry tests also showed that the increase in HMW-GS increased shear viscosity, which decreased rapidly in the absence of HMW-GS. With the effects of glutenin-to-gliadin ratio removed, these results still support previous results (where the glutenin-to-gliadin ratio was different) showing the HMW-GS loci differ in their contribution to dough in the order of *Glu-D1* > *Glu-B1* > *Glu-A1*, and that this relationship is independent of protein content.

## CONCLUSIONS

The effects of glutenin-to-gliadin ratio and the presence of certain glutenin subunits were separated. Both these parameters contributed independently to dough characteristics. At uniform protein content, the differences between samples of this set were greatly reduced when the glutenin contents were corrected. While not as dramatic as in the uncorrected results, there were significant differences in mixing times (indicative of dough strength) between the null lines containing 0 or 1 locus and those containing 2 or 3 loci coding for HMW-GS. These results may be explained in part by the increased HMW-GS to LMW-GS ratio in samples containing higher numbers of HMW-GS coding loci and in part by an increase in average polymer size where all HMW-GS encoding loci were present in the flour.

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