JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Dynamic Viscoelastic and Tensile Properties of Gluten and Glutenin Gels of Common Wheats of Different Strength

CHIA C. LEE AND STEVEN J. MULVANEY*

Department of Food Science, Cornell University, Ithaca, New York 14853

Dynamic viscoelastic properties at 25 °C of gluten and glutenin gels were obtained from Canadian common wheats of different strengths. The relaxation spectra showed a maximum intensity at a characteristic relaxation time (τ^*). The relaxation modulus associated with this maximum was taken as the strength of the glutenin or gluten gel transient network ($G(\tau^*)$). The ratio of $G(\tau^*)$ for glutenin and gluten gels from the same cultivar ranged from 5.6 for an extra strong cultivar to 51.1 for a soft wheat. This gives indirect evidence that the gliadin fraction weakens the glutenin gel network more in weaker cultivars. In addition, the fact that both glutenin and gluten gels showed extensive stress relaxation coupled with the fact that addition of L-cysteine to a gluten gel eliminated the network structure at 25 °C and resulted in a power law stress relaxation spectrum suggests that the transient network in gluten is a reversible network. This power law relaxation pattern was not seen here for an entangled polymer melt (poly(dimethylsiloxane)). It was also found here that the viscosity of the gluten gel ($G(\tau^*) \times \tau^*$) trended best with the tensile stress build-up in a uniaxial tensile test of gluten gels. Together, these results indicate that both network strength and relaxation times should be considered in characterizing the linear viscoelastic properties of hydrated cereal proteins.

KEYWORDS: **Gluten; glutenin; reversible gels; rheology; stress relaxation; viscoelastic properties; tensile properties**

INTRODUCTION

During the mixing of wheat flour and water, gluten is developed and is generally assumed to form a continuous protein network structure within the dough. However, in the context of polymer gels and gelation, the word "network" has a special meaning. Its use implies that the effective degree of cross-linking is greater than the critical degree of cross-linking, which denotes the sol to gel transition (*1*). This is true regardless of whether the gel is a chemical or physical gel. For the latter, the effective degree of cross-linking at a fixed temperature is a function of polymer concentration. The concentration of glutenin protein in a typical bread dough is only about 5%. This low concentration combined with the fact that the highest molecular weight glutenin subunits (HMW GS) represent only a fraction of the total glutenin proteins (∼25% for one particular good bread-making cultivar (*2*)) and that their MW is not that high (∼80 000−133 000 (*3*)) leads one to ask whether there is actually a true protein network in bread doughs. If doughs are not strictly gels, then what are they? The same question could be asked of gluten itself.

Doughs have been referred to as viscous liquids, but in this context, the term "liquid" must refer to a sol, as there are undoubtedly clusters of some sort in the hydrated gluten proteins in a dough. A sol near the gel point would be expected to have a very large viscosity, since the viscosity goes to infinity at the gel point. The critical gel (i.e., at the gel point) represents the transition from sol to gel and can be recognized by its power law stress relaxation (SR) pattern that persists over several decades of time (or frequency) in the terminal region (*1*, *4*). The appearance of this power law relaxation pattern in the terminal region of viscoelastic behavior allows one to distinguish sols from linear viscoelastic polymer melts, which are characterized by a single longest relaxation time in the terminal zone. Thus, the terminal zone is defined by a polymer's longest relaxation time, and for times longer than this relaxation time (or frequencies lower than its reciprocal value), the polymer can be considered to be a viscous liquid, albeit one with a very high viscosity.

We have hypothesized that some of the mystery surrounding doughs and gluten can be lifted by considering certain glutenin proteins as associating polymers. Synthetic associating polymers are formed by grafting noncovalent functional groups onto linear amorphous polymers, often just at the ends (a process termed end-linking). Rubinstein and Dobrynin (*5*) discuss some general aspects of associating polymers and thermoreversible gelation. They note that intermolecular interactions due to the presence of noncovalent functional groups lead to very high viscosities at low concentrations, making these polymers useful as adhesives and thickeners. At higher concentrations, associative polymers aggregate into clusters and if the concentration is high enough they spontaneously form reversible gels. This unusual

* To whom correspondence should be addressed. Tel: (607)255-7921. Fax: (607)254-4868. E-mail: sjm7@cornell.edu.

10.1021/jf020719l CCC: $25.00 © 2003 American Chemical Society
Published on Web 03/12/2003

mechanism of thermoreversible gelation does not require heating or addition of coagulants or cross-linking agents. The term "thermoreversible gelation" in the context of associating polymers refers to the fact that the cross-links in the reversible network break and reform continuously. The rate of bond breaking is temperature-dependent and so is characterized by an activation energy (*5*). Thermoreversible gelation is distinctly different from the melting of so-called physical cross-links with an increase in temperature and subsequent reformation of the physical cross-links upon cooling, e.g., as seen with a gelatin gel. In the latter case, the physical cross-links are essentially permanent below the melting point and disappear above the melting point. Thus, it makes sense to refer to the dynamics of a reversible network or gel, while a physical gel can be described by its frequency-independent elastic modulus. According to Tanaka and Edwards (*6*), "The most striking natural characteristic of a reversible network is its internal fluidity; each chain can diffuse through the entire network due to the finiteness of the junction lifetime, despite being partially connected to the macroscopic network structure in the course of movement." These authors go on to say that such a system can therefore flow under an external stress on a longer time scale than the junction lifetime. The basic dynamic quantities of a more concentrated reversible network well above the gel point are its longest relaxation time (τ_{max}) and the viscosity (η). Here, τ_{max} represents the finite lifetime of the reversible cross-links, which for an ideal, uniform, reversible network will be a single value (*5*). Although not stated explicitly by the above authors, it seems that for real associating polymer systems a weak reversible network that flows under gravitational stress would become a reversible gel if the polymer concentration is increased beyond a critical point (defined as the same material at a high enough concentration of polymer so that it no longer flows under gravitational stress in a practical time frame). In this case, the lifetime of the reversible bonds is greater than the relaxation time of the network strands, and so, the breaking and reforming of these reversible cross-links dominate the dynamic linear viscoelastic properties (*5*). This latter description fits well with the physical state of the gluten and glutenin materials evaluated here, and so, they will be referred to as reversible gels. Leibler et al. (*7*) discuss the case where the lifetime of the reversible cross-link is less than the terminal relaxation time of a polymer melt; thus, the conventional terminal zone viscous flow process was slowed, a process referred to as "sticky reptation", but the system was still basically an entangled polymer melt and not a gel.

The above description of associating polymers and reversible networks fits very well with what is known of the chemistry of the glutenin proteins and the rheological properties of the hydrated cereal proteins. It follows that determining the dynamic linear viscoelastic properties of gluten gels could provide the basis for evaluating gluten quality based on a fundamental measurement of its inherent gluten network strength and viscosity. We have proposed in earlier work that at least some of the covalent disulfide bonds in doughs act as reversible cross-links due to the presence and mobility of free thiols in hydrated gluten gels and doughs (*8*). This allows these covalent chemical bonds to undergo interchange reactions and act as reversible bonds.

Thus, we hypothesize here that a number of the glutenin polypeptides in glutenin and gluten gels are natural associating polymers that undergo spontaneous gelation when wheat proteins are mixed with water near room temperature. We suppose that gluten gels represent a single phase but contain a sol fraction and a separate reversible gel network. Gluten gels have not been considered in the context of a thermoreversible gelation process to the best of our knowledge, probably due to the absence of an obvious sol to gel transition as is found with chemical or physical gels. However, we believe that we have demonstrated the thermoreversible nature of the gluten gel network by selective addition of L-cysteine (L-Cys) to gluten, which caused the network to essentially disappear although the temperature was the same as for the control gluten. The main point of this work then is to provide extensive experimental data in support of this hypothesis, in particular how SR allows one to use time to separate out the effects of the sol (unconnected) and reversible network fractions on the dynamic LVP. We will show that the very idea of network strength of a reversible gel (unlike a chemical gel or physical gel below the melting point) is inherently a dynamic property and not a static property. We will also show a strong relationship between the viscosity of gluten gels of different strength and their tensile stress during stretching at a constant rate. It may seem contradictory to refer to the viscosity of a self-standing gel, but as explained above, this viscosity represents the internal fluidity of the network and at low frequencies is governed by the lifetime of the reversible bonds (*5*).

MATERIALS AND METHODS

Five bread wheat flour samples from the 1997 Canadian Cooperative Test, the final stage of Canadian wheat breeding trials, were provided by the Grain Research Laboratory, Canadian Grain Commission (MB Canada) for use in this study. They included two Canada Western Extra Strong (CWES) cultivars: Glenlea (Gle) and experimental line ES 12; one Canada Western Red Spring (CWRS) cultivar: Neepawa (Npw); one Canadian Prairie Spring-Red (CPS-R) cultivar: Crystal (Cry); and one Canadian Prairie Spring-White (CPS-W) cultivar: HY 442. The protein content on a 14% moisture basis is given after the abbreviation of the cultivar name below. Gluten and glutenin gels were evaluated for Gle (12.8%), Npw (14.4%), Cry (11.0%), and HY 442 (11.4%). Because of insufficient flour, only gluten gels from ES 12 (12.8%) were tested.

Gluten gels obtained from two soft white spring wheats (SWS), the experimental line SWS 231 (9.8%) and Reed (10.6%), also provided by the Grain Research Laboratory, Canadian Grain Commission (MB Canada), were included in this study. Chemical, dough-mixing curves, physical dough properties obtained via extensigraph and alveograph, and baking quality for many of these cultivars (or similar cultivars from the same classification) have been reported previously (*9*, *10*). However, none of those chemical or physical characterizations provides a direct measurement of the strength of the network in dough or gluten, which can only be obtained from rheological measurements in the linear region.

In addition, the SR behavior of bulk poly(dimethylsiloxane) (PDMS) was determined as a control representative of an entangled polymer melt in the terminal zone. The PDMS sample used here was a product of General Electric (GE; SE30, GE silicones; www.gesilicones.com) that is used for calibration of the Bohlin VOR-M rheometer. According to a representative of GE, the approximate number average molecular weight of SE30 PDMS is 385 000. PDMS is a well-characterized synthetic polymer, which exhibits the classical viscosity $\propto M^{3.4}$ relationship for entangled polymer melts (*11*). The PDMS sample used here flowed readily at 25 °C under gravitational stress, while the gluten and glutenin samples did not. These observations suggested that the hydrated cereal samples were more gellike than liquidlike at 25 °C. It will be interesting to see how the SR patterns reflect these visual observations.

Finally, a different batch of Gle flour (denominated Gle*) was used to determine the effect of l-Cys addition. The distinction between Gle* and Gle is necessary on account of possible variations between flour samples of different crop years. L-Cys was obtained from Sigma Chemical Co. (St. Louis, MO). The SR behavior of hydrated gliadin

(Sigma Chemical Co.) with and without added L-Cys was also determined. It was expected that gliadin would represent a type of material intermediate between PDMS and gluten and also serve as a control for the short time relaxation behavior of gluten.

Extraction and Fractionation of Gluten Proteins. AACC method 38-10 (*12*) was used to obtain wet crude gluten from mixed doughs with slight modification. After it was mixed, the dough was immersed in distilled water at room temperature for 20−30 min before being washed and was then freeze-dried. The freeze-dried material was ground (Arthur C. Thomas Co., Philadephia, PA) through a mesh size of 60 to obtain samples of similar physical size for rehydration. Freeze-drying and rehydration were considered the only feasible means of obtaining gluten and glutenin gels of the same moisture content for comparison. Lipids were not extracted, as they would be part of the natural gluten in a mixed dough and should be included in a measure of the natural gluten network strength.

Osborne Glutenin Fraction. Glutenin was obtained from freeze-dried crude gluten as the fraction insoluble in 70% ethanol. Freeze-dried gluten powder was mixed with 70% ethanol to obtain a 20% solids slurry. The sample was mixed on a stir plate for 12 h and centrifuged (Sorvall RC-5B Refrigerated Superspeed Centrifuge, Kendro Laboratory Products, Newton, CT) at 10 000 rpm and 25 °C for 20 min. The alcohol was then decanted, and fresh 70% alcohol was added again. The samples were then thoroughly mixed and centrifuged again under the same conditions. The extraction was then repeated two more times using 5000 and 2000 rpm, respectively. The remaining alcohol was washed out of the glutenin samples using distilled water. Glutenin was freeze-dried and ground following the same procedure for glutens.

The Osborne glutenin fraction is sometimes further fractionated into glutenin soluble in dilute acid and a residual gel protein (*2*, *3*). In this context, our glutenin fraction would contain the so-called gel protein fraction or sodium dodecyl sulfate (SDS) insoluble protein as well. Khatkar et al. (*2*) observed some small glutenin polymers in their unreduced Osborne gliadin fraction on SDS−polyacrylamide gel electrophoresis (PAGE) and also saw the expected lowest molecular weight (LMW) GS (MW 34 000−45 000) on the reduced extracts on SDS−PAGE. Thus, it is important to emphasize that differences between the viscoelastic properties of our glutenin gels and the viscoelastic properties of gluten gels for the same cultivar would presumably include the effects of both monomeric gliadins and LMW glutenins soluble in 70% ethanol, as well as major nonprotein components such as lipids.

Rheological Measurements. Gluten and glutenin gels at 60% water on a total weight basis (% MC) were prepared from freeze-dried powders by addition of calculated amounts of distilled water. For Cys-treated Gle* glutens, L-Cys was dissolved in the water prior to mixing to obtain final concentrations of 125, 200, and 250 ppm in the gluten gel. The samples were pressed between two metal plates with a 2.5 mm gap and allowed to relax for 1 h to form sheets of uniform thickness. A small amount of mineral oil was used on the plates to prevent sticking and tearing of the sheet upon removal from the plates after resting. The use of this oil is not an ideal situation, and further work is warranted to develop a sample preparation procedure that does not require its use. Extracted gliadins from the different cultivars were not characterized here. Instead, a gliadin sample was prepared at 50% MC by hand-mixing Sigma gliadin with distilled water. Because of the stickiness of the gliadin samples, they were placed directly on the rheometer lower plate after mixing. The main purpose of including the gliadin resin sample was as a control for the short time relaxation of gluten gels. The significant difference between the relaxation pattern of gluten gels and the gliadin alone at short times would suggest that gliadin and glutenin proteins are compatible and form a single phase. In other words, the relaxation spectrum of gluten gels is not just the gliadin spectrum followed by the glutenin spectrum. The % MC was a little lower for the gliadin (50% MC vs 60% MC for the gluten) due to differences in their water absorption properties.

SR. SR experiments were done with a Bohlin VOR-M rheometer (Bohlin Instruments, Cranbury, NJ), using parallel plate geometry (25 mm plate diameter and 2.5 mm plate gap) in shear mode. Samples were cut from the gluten or glutenin sample sheets using a 25 mm diameter cylinder with sharpened edges.

The disc-shaped sample was glued to the lower plate of the rheometer, and an upper serrated 25 mm plate was used to minimize slippage. The exposed edge of the sample was covered with a thin layer of mineral oil and a moisture trap placed around the sample to minimize moisture loss during measurements. After it was loaded in the rheometer, all samples were allowed to relax for a minimum of 60 min. Measurements were made with a 1.483 g cm torsion bar for gluten and gliadin and a 90.320 g cm torsion bar for glutenin. The shear strain was 0.5%, and the rise time for the applied strain was 0.2 s in all cases. This strain was within the linear viscoelastic region of hydrated gliadin and the gluten and glutenin gels as determined in our laboratory previously. The relaxation modulus ($G(t)$) was obtained for 10 000 s at 25 °C for all samples.

The SR spectra was calculated by the Bohlin software from the relaxation curves using the first-order approximation or Alfrey's rule (*13*):

$$H_{(\tau)} = - \left.\frac{\mathrm{d}G(t)}{\mathrm{d}\ln t}\right|_{t=\tau} \qquad (1)$$

The initial portion of the relaxation spectrum was cut off from the point where it began to fall to zero on the left. This approach was used previously by Cunningham and Hlynka (*14*) for wheat flour doughs. We determined the SR spectra here experimentally, since it was clear that gluten and glutenin gels did not exhibit the classical (i.e., theoretical) linear viscoelastic behavior of entangled polymers. This precludes the use of linear viscoelastic theory to calculate one viscoelastic function from another for these materials.

Tensile Testing. Tensile properties of gluten gels were determined using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, U.K.). Rectangular strips of 50 mm long (L_0) and 20 mm wide were cut from the gluten sample sheet (2.5 mm thickness). In the middle of the strip, 1 mm notches were cut, one on each side, to help prevent tearing of the sample at the grips. Tensile stress (tensile force divided by initial cross-section area) of gluten strips was determined as a function of time at a constant crosshead speed of 0.1 mm/s. A Plexiglas humidity chamber was constructed and placed around the sample in the TA-XT2 to minimize moisture loss of gluten samples during the test. Humid air was directed into the chamber through a side opening using a humidifier. The temperature inside the chamber was maintained at a constant 25 °C, and the relative humidity (RH) was kept around 95%, which was essentially the RH of the wet gluten samples.

Statistical Analysis. All gluten gels and gliadin were tested in triplicate using independent samples. Four samples were used for glutenin testing, since we had less experience with these samples. A two-tailed *t*-test was used to determine statistical significance for the mean values of certain parameters obtained from the SR experiments (*15*). The error bars in SR figures represent the standard error of the mean.

RESULTS AND DISCUSSION

Results will be presented in four general sections. The first will be our analysis of the SR patterns for Gle* gluten gel and the effect of L-Cys on its relaxation patterns. If there are reversible disulfide cross-links in gluten gel, addition of L-Cys at constant temperature should reduce the effective degree of cross-linking and weaken the gluten gel network. A graphical/analytical procedure will then be used to compare results for gluten gels and glutenin gels from cultivars of different wheat classes and mixing strength. This will give indirect evidence of the effect of the gliadin fraction on the strength of the glutenin network for these cultivars. We will also comment on the similarity between the SR patterns seen here for glutenin gels and the glutenin size distribution patterns previously published. This is good evidence that SR patterns in the linear viscoelastic region can be considered as the kind of "molecular rheology" described by Marin and Montfort (*16*). Finally, the patterns in the tensile stress of gluten gels during stretching will be

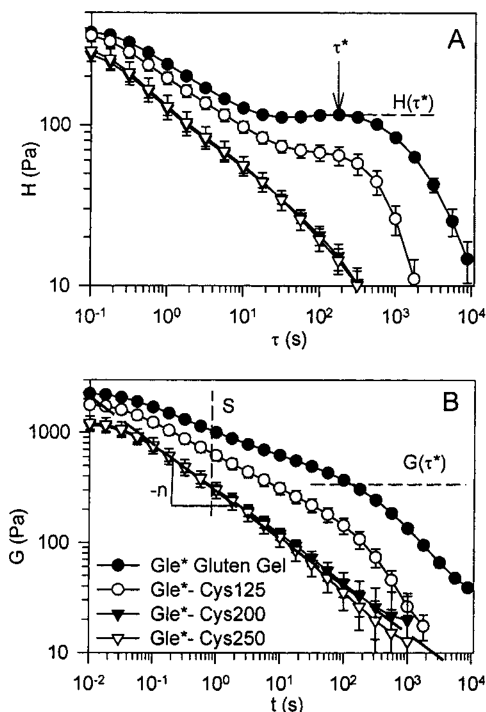

Figure 1. Effect of added L-Cys (125, 200, and 250 ppm) on the SR spectra (**A**) and the corresponding relaxation modulus (**B**) of Gle* gluten gel as compared to control Gle* (no added L-Cys). Results are for 60% water on total weight basis gels at 25 °C and a shear strain of 0.5%.

compared to those for SR of the same gels. This will provide a linkage between the LVP and the large deformation properties of gluten gels.

Characterization of the Gluten Gel Relaxation Curves and Relaxation Spectra. SR results are shown in **Figure 1A** (relaxation spectrum, $H(\tau)$ vs τ) and 1B (relaxation modulus, $G(t)$ vs t) for the Gle* gluten gel with and without added L-Cys. The effect of added L-Cys on these SR patterns will be discussed later in this section. Characterization of the relaxation patterns results in two sets of parameters: one related to the pattern of $H(\tau)$ itself and the other related to the corresponding $G(t)$ curve. $H(\tau)$ for Gle* gluten gel without added L-Cys showed two distinct relaxation modes. A power law relaxation mode was observed for about the first 10 s, followed by a broad nonpower law relaxation, which contained an inflection point characteristic of transient networks. This inflection point in the spectrum was denoted by its intensity ($H(\tau^*)$) and its relaxation time (τ^*). The presence of two distinct relaxation processes in gluten gels is well-known (*17*); however, our analysis and interpretation of these SR results is unique.

The peak, or inflection point in the relaxation spectrum, which is essentially the derivative of the $G(t)$ vs time curve on logarithmic coordinates, of each experimental curve was determined by graphical analysis of the spectrum and the values averaged. The inflection point was determined as that point at which the rate of decrease in the spectrum increased noticeably relative to the preceding nearly linear section of the spectrum on logarithmic coordinates. This point was then used to locate the corresponding inflection point in the relaxation curve as shown in **Figure 1B**. Determination of inflection points is more ambiguous than peaks, and we clearly indicate that this procedure (peak or inflection point) leads to an approximation of the true pseudo-elastic modulus $G(\tau^*)$.

We interpret $G(\tau^*)$ as a characteristic "base network strength" of the gluten gel associated with the longest relaxation mode, τ^*. This idea is analogous to the model of Rubinstein and Semenov (*18*) where the dynamic elastic modulus (G') of a reversible polymer gel can be pictured as having two main contributors, Gstr and Gnet. The more important one is Gnet, which accounts for the network elasticity and breakage of strands within this reversible elastic network (*18*), while the other term accounts for contributions to the relaxation modulus due to free clusters and relaxation modes of strands within the network.

Table 1. SR Curve and Spectra Parameters for Gle* Gluten, Gle* + 125 ppm L-Cys (Gle*–Cys125), Gle* + 200 ppm L-Cys (Gle*–Cys200), and Gle* + 250 ppm L-Cys (Gle*–Cys250)

	$G(t)$ parameters			$H(\tau)$ parameters	
	n	S (Pa s^n)	$G(\tau^*)$ (Pa)	τ^* (s)	$H(\tau^*)$ (Pa)
Gle*	0.21	1015	323	153	116
Gle*–Cys125	0.30	619	144	96	68
Gle*–Cys200	0.41	299	NA	NA	NA
Gle*–Cys 250	0.41	301	NA	NA	NA

In addition, we obtained the strength (S) and relaxation exponent (n) of the power law relaxation mode using eq 2 (*19*). Equation 2 represents the CW (Chambon−Winter) power law relaxation mode associated with the critical gel. We have used this relationship in a more general way to describe the initial power law relaxation mode for all of our gluten gels.

$$G(t) = St^{-n} \tag{2}$$

Rodd et al. (*20*) commented that no fundamental molecular meaning needs to be assigned to S, but it could be used for comparative purposes. We used it here because the word "strength" is often used in the context of gluten gels and doughs, without being precisely defined. We are not aware of anyone discussing or using the concept of the critical gel for reversible gels, although it is clear that there is a critical concentration of associating polymer (or effective degree of cross-linking) needed to obtain the sol to gel transition for associating polymers. A possible interpretation of S here would be the "short time" strength of the gluten gel, which decreases to a limiting lower value as L-Cys is added (to be discussed next). The short time strength would include contributions from the unrelaxed network ($G(\tau^*)$) plus unconnected clusters with relaxation times greater than about 1 s.

Effect of Added L-Cys. Addition of L-Cys at 25 °C had a dramatic effect on the relaxation spectrum and relaxation modulus of Gle* gluten gel as shown in **Figure 1A**,**B**, respectively, and **Table 1**. For Gle*−Cys125 gluten gel, the power law spectrum was extended to longer relaxation times, and the intensity and relaxation time at the inflection point were dramatically reduced (116 to 68 Pa and 153 to 96 s, respectively). It was quite surprising to see that the effects of 200 and 250 ppm added L-Cys to Gle* gluten gel were essentially the same. This implies that a saturation of some important reversible cross-linking phenomena may occur at higher concentrations of L-Cys. Further addition of L-Cys would be needed to determine if this is indeed the case. In addition, both the spectra and the relaxation curves showed extended power law relaxation for these two levels of added L-Cys. This relaxation behavior is characteristic of near-critical physical and chemical gels. We interpret these findings to mean that addition of L-Cys reduced the effective degree of cross-linking in gluten such that the reversible gel network was completely eliminated at 25 °C, all other factors being the same, in this time frame. Apparently, a number of critical cross-links were lost revealing a power law

relaxation pattern. This unusual relaxation pattern has been attributed to the presence of a distribution of sizes of self-similar branched clusters at the gel point (*21*). In this context, "loss of reversible cross-links" means that their effective lifetimes become shorter than the accessible times in SR upon addition of L-Cys.

The $G(t)$ parameters were also affected by added L-Cys as shown in **Table 1**. S decreased from 1015 Pa s^n to 619 Pa s^n upon addition of 125 ppm L-Cys and then to about 300 Pa s^n for the higher levels of added L-Cys. The relaxation exponent (n) increased from 0.21 to 0.30 and then to a constant value of 0.41 for the addition of the higher levels of L-Cys. $G(\tau^*)$ decreased from 323 to 144 Pa for the addition of 125 ppm L-Cys and then effectively disappeared for the two higher concentrations. Thus, the Gle* limiting values of the power law relaxation parameters have been obtained as $S = 300$Pa s^n and $n = 0.41$. For a chemically cross-linked PDMS critical gel in the absence of entanglements, S was 226 Pa $s^{1/2}$ and n was 0.5 (*4*), similar to the values found here for the gluten. It cannot be determined for sure from this work alone whether the power law relaxation phenomena seen here for gluten gels have the same interpretation as for bulk chemical gels at the gel point. In the latter cases, a transition from viscoelastic liquid to the critical gel and then to the viscoelastic solid state was demonstrated as the cure time was increased. We did not observe a relaxation pattern characteristic of a simple viscoelastic liquid, such as PDMS, which may be due to the added complexity of gluten relative to simpler monodisperse synthetic cross-linking polymers. An important observation here is that the network strength ($G(\tau^*)$) can only be resolved from the power law relaxation mode at longer times, with values of τ^* for the gluten gels to be discussed here being in the hundreds of seconds. These low frequency (long time) relaxation modes cannot be accessed by dynamic frequency sweeps at 25 °C for gluten gels. We have pointed this out previously, but many investigators still characterize the linear viscoelastic properties of gluten and glutenin gel networks and doughs using small amplitude oscillatory testing over just two decades of frequency, 0.1−10 Hz. This testing procedure clearly only probes relaxation modes in the power law relaxation region.

Comparison of the relaxation curves for the gluten gels shown in **Figure 1B** with the dynamic moduli data (five decades of shifted frequency), of Chambon and Winter (*4*) for their PDMS chemical gels at different degrees of cross-linking suggests that the short time (high frequency) power law relaxation mode is common to both reversible and chemical gels near the gel point. The presence of a power law spectrum at the gel point has also been demonstrated for a physical cross-linking PDMS system (*22*). In this case, linear PDMS was converted to an associating polymer by the addition of carboxyl functional groups along the chain, thus allowing for hydrogen bonding. The sol to critical gel to solid transition was achieved by heating (curing) for up to 18 h at elevated temperature. The difference between the physical and the chemical gels described above and the gluten gel is that the power law spectrum was revealed for the gluten gel by the addition of L-Cys at a temperature below that required for physical cross-linking of gluten, which begins to occur at about 55 °C (*23*). Thus, the cross-links responsible for the gluten gel network, as determined by $G(\tau^*)$, are not permanent, as with a chemical cross-linking system, and are not typical physical cross-links as the temperature was the same for the control gluten and those with L-Cys added. It seems that the only logical conclusion is that the cross-links responsible for the network are reversible cross-links.

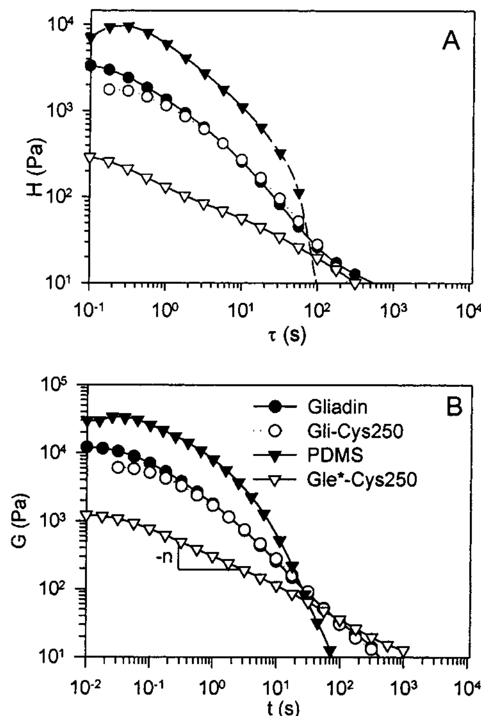

Figure 2. SR spectra (**A**) and the corresponding relaxation modulus (**B**) of hydrated gliadin (50% water on total weight basis), gliadin + 250 ppm L-Cys (Gli–Cys250) as compared to bulk PDMS and Gle* gluten gel (60% water on total weight basis) + 250 ppm L-Cys (Gle*–Cys250). The temperature was 25 °C, and the shear strain was 0.5% for all materials.

Thus, the power law relaxation mode seen here might represent relaxation modes due to the presence of self-similar glutenin clusters that are not connected to the reversible gel network. In this case, self-similar may mean clusters of different size, that is, with differing number of polypeptides but with the same proportions of HMW and LMW subunits as reported by Graveland et al. (*3*) for the 5 + 10 cultivar Sicco. As with synthetic reversible networks, the concept of the "strength" of a reversible network becomes one of connectivity (*24*), which is more difficult to define at the molecular level for associating polymers than for chemical cross-linking polymers. The more precursor polymers attached to the network, the stronger is the network. In our case, we have identified two strengths for gluten gels, a short time strength (S) that may be relevant to fast dough processes such as mixing and a longer time network strength ($G(\tau^*)$), which may be more relevant to slower dough processes such as oven rise or proofing in bread making. This will be discussed further in the viscosity section below.

Relaxation Patterns of Viscoelastic Liquids. The uniqueness of the near critical power law relaxation mode discussed above can be appreciated by comparison to the relaxation patterns for hydrated gliadin (Gli), Gli + 250 ppm L-Cys (Gli−Cys250) and uncross-linked PDMS as shown in **Figure 2**. PDMS is a well-characterized synthetic polymer that does not have any associating functional groups or physical cross-links. It readily flows at 25 °C since its Tg is about −123 °C. Gliadin is much more complex than PDMS but not as complex as gluten gels. It is generally accepted that disulfide linkages in gliadin are mainly intramolecular, while they are intermolecular in glutenin. Hydrated gliadin looks and feels different from the PDMS sample and the gluten gels. It is more like a sticky resin. The Gli−Cys250 relaxation results were superposed with the Gli results very well by shifting 0.5 decades in time. No vertical shift factor was needed since there was no change in concentra-

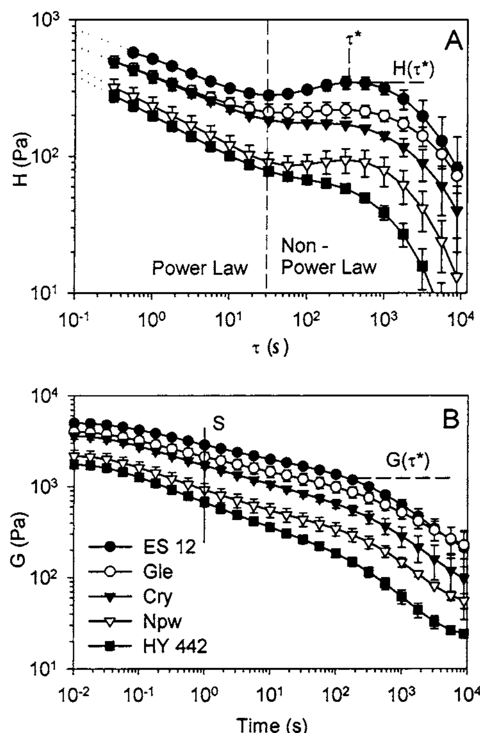

Figure 3. SR spectra (**A**) and the corresponding relaxation modulus (**B**) for five gluten gels (60% water on total weight basis) from bread wheat cultivars of different classes: ES 12 (CWES), Gle (CWES), Cry (CPS-R), Npw (CWRS), and HY 442 (CPS-W). The temperature was 25 °C, and the shear strain was 0.5% for all materials.

tion of gliadin. This indicates that the effect of added L-Cys on gliadin was thermorheologically simple.

The undiluted PDMS had the highest $G(t)$ value initially but rapidly relaxed stress eventually crossing over the slower relaxing Gli and Gli−Cys250 relaxation curves after about 30 s. PDMS shows the classic terminal zone relaxation on log−log coordinates of a linear viscoelastic liquid with a single characteristic terminal relaxation time. Gliadin shows an extended relaxation pattern as compared to PDMS but is also different from that of the Gle* critical gel. Gliadin relaxation dynamics probably represent an intermediate behavior, where terminal zone relaxation modes are affected by the "sticky reptation" process described by Leibler et al. (*7*), but these secondary associations are probably uniformly distributed and do not form clusters or reversible network structures as with the gluten gels. This also implies that HMW GS are needed for the development of such structures, since these subunits are only found in gluten or glutenin gels. Similar terminal zone behavior to gliadin has been reported for starch aqueous systems and was attributed to extensive hydrogen bonding in that system (*25*). It is also worth noting that viscoelastic liquids are characterized more by fast relaxation of stress than their short time or high frequency modulus. Thus, it may be difficult to distinguish viscoelastic liquids, reversible networks, and physical gels from each other based on frequency sweeps alone. This is another demonstration of the importance of using long time or low frequencies to determine the permanence of any intermolecular interactions or cross-links contributing to the high frequency viscoelastic properties.

SR Patterns of Gluten Gels of Different Mixing Strength. **Figure 3** shows the relaxation spectra (**3A**) and relaxation modulus (**3B**) for five gluten gels from bread wheat cultivars of different mixing strengths. In general, the relaxation patterns

Table 2. SR Modulus ($G(t)$) and Spectra ($H(\tau)$) Parameters for Gluten Gels (60% Water on Total Weight Basis) from Common Wheat Cultivars Representing Different Canadian Wheat Classes[a]

	$G(t)$ parameters			$H(\tau)$ parameters	
	n	S (Pa s^n)	$G(\tau^*)$ (Pa)	τ^* (s)	$H(\tau^*)$ (Pa)
ES 12 (CWES)	0.15	2844	913 ± 64 a	434 ± 99	356 ± 36
Gle (CWES)	0.16	2105	784 ± 169 a,b	327 ± 90	227 ± 31
Cry (CPS-R)	0.20	1694	536 ± 53 b	193 ± 15	173 ± 5
Npw (CWRS)	0.22	908	242 ± 38 c	318 ± 37	94 ± 24
HY 442 (CPS-W)	0.27	665	145 ± 13 c	178 ± 11	63 ± 2
SWS 231 (SWS)	0.28	684	138 ± 9 c	170 ± 29	68 ± 6
Ree (SWS)	0.35	648	101 ± 48 d	140 ± 22	58 ± 6

[a] Numbers with the same letters in the same column denote a statistically insignificant difference at 95% confidence.

for all of these gluten gels are similar to that of the Gle* gluten but with different values of τ^*, $H(\tau^*)$, n, S, and $G(\tau^*)$ as shown in the top of **Table 2**.

As seen in **Table 2**, there is a noticeable drop off in the strength of the power law relaxation mode and $G(\tau^*)$ for the HY 442, SWS 231, and Reed gluten gels relative to the next strongest cultivar, Neepawa. On the other hand, there is a noticeable increase in S values for cultivars above Neepawa in **Table 2** and a decrease in n. $G(\tau^*)$ and $H(\tau^*)$ values also step up for cultivars above Neepawa. Thus, the gluten gel characteristics of Neepawa appear to represent a transition between naturally weak and naturally strong cultivars, with Neepawa being moderately strong. These data are consistent with the fact that Neepawa represents the minimum quality standard for the CWRS class of wheats. This also suggests that the rheological properties of the gluten gel as described here have potential as a grading scheme for wheat cultivars.

SR Patterns of Gluten Gels of Soft Wheats. **Figure 4** shows SR patterns for the two soft wheat cultivars Reed and SWS 231 along with HY 442, the weakest bread wheat cultivar in our study, while SR parameters are reported at the bottom of **Table 2**. The results show that all three of these gluten gels exhibit similar and relatively weak characteristics at short times (higher n and lower but similar S values as seen in **Table 2**).

It was interesting to see that the values for n, S, and $G(\tau^*)$ were essentially identical for the HY 442 (medium hard) and SWS 231 gluten gels when compared at the same concentration (60% MC in both cases). This suggests that at least some of the functional differences between these two types of wheat are related to the lower overall protein content in soft wheat doughs. Also, it was observed that the values for S and $G(\tau^*)$ for HY 442 were essentially identical to those of Gle*−Cys125. A similar result was seen in our previous work when L-Cys was added to an extra strong dough from cultivar ES 12 (*9*). This supports the view that the "extra strength" of Gle* gluten gels could be due to its naturally higher glutenin content and therefore higher concentration of associating polymers relative to HY 442 but that this natural state of affairs can be shifted by selective addition of L-Cys to reduce the effective degree of cross-linking. Statistical analysis indicated that $G(\tau^*)$ was not significantly different for Gle (CWES) and Cry (CPS-R) cultivars or for Npw (CWRS), HY 442 (CPS-W), and SWS 231 (SWS) but that there were four distinct groupings based on $G(\tau^*)$. This classification of wheat cultivars based on statistical differences in $G(\tau^*)$ would lead to different groupings of cultivars than the statutory Canadian wheat classes, which are based on visual distinctions between wheat kernels. This suggests that there are two basic types of wheat quality, one related to quality of the kernels themselves and their visual

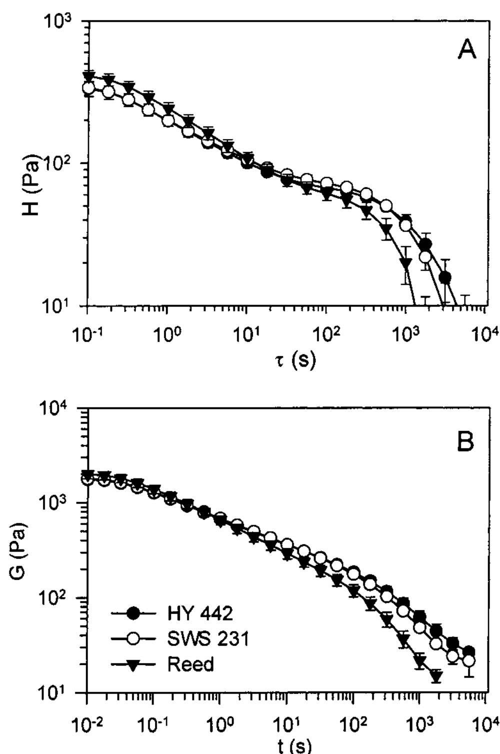

Figure 4. SR spectra (**A**) and the corresponding relaxation modulus (**B**) for gluten gels (60% water on total weight basis) from HY 442 (CPS-R) and SWS wheat cultivars SWS 231 and Reed. The temperature was 25 °C, and the shear strain was 0.5% for all materials.

Table 3. Selected Chemical and Physical Dough Properties for Cultivars Representing the Following Canadian Wheat Classes: CWES, CWRS, CPS, and Canada Western SWS[a]

	class	mixing time (min)	ratio of gliadin to glutenin	unextractable protein (%)
ES 12	CWES	6.1	0.53	25
Neepawa (Npw)	CWRS	3.2	0.72	16
HY 443	CPS	2.8	0.76	12
SWS 238	SWS	1.5	0.60	11

[a] Data from Edwards et al. (2001).

characteristics and the other related to the network strength of the hydrated cereal proteins.

Relationships between Linear Viscoelastic Properties and Chemical and Functional Properties. Chemical composition, physical dough properties, SR patterns for doughs, and baking performance have been reported previously for Gle, ES 12, Npw, and HY 442 (*9*), while creep results and gliadin and glutenin ratios have been reported previously for ES 12, Npw, HY 443, and SWS 238 (*10*). Lukow et al. (*26*) reported that the HMW subunit composition of Glenlea and Neepawa was 2*, 7 + 8, 5 + 10 and 1*, 7 + 9, 5 + 10, respectively. Their results also indicate that Canadian soft wheats are very likely to be 2 + 12 allele types. Previously, the ratio of gliadin to glutenin was found to be 0.53 for ES 12, 0.72 for Neepawa, 0.76 for HY 443 (somewhat stronger dough rheology than the HY 442 used here), and 0.6 for a SWS experimental soft wheat similar to the SWS 231 used here (*10*). The unextractable proteins (referred to as gel protein) were included in the glutenin fraction for these calculations and were 25% for ES 12, 16% for Npw, 12% for HY 443, and 11% for SWS 238. These data are summarized in **Table 3**. Thus, one can see that there is a clear trend between the gel network strength ($G(\tau^*)$) and the unextractable protein. One would expect unextractable protein to be highly cross-linked, thus rendering it insoluble. This is different from saying it is a high molecular weight material with entanglements, since in the context of gelation one talks of the weight fraction of cross-linked gel protein and not its molecular weight. A molecular weight distribution only makes sense for unconnected clusters, which are in the sol (extractable) protein fraction (*27*). Clearly, one could also have cross-linked clusters in the sol fractions and flow of a dough, thus cross-linking and flow are not contradictory as it might seem at first.

The glutenin content of Gle was higher than for Npw, but their HMW subunit composition was similar. All of the parameters of the relaxation modulus and spectrum indicate a much stronger (better connected) sol and gel network for the Gle gluten gel. This result may be expected, as the concentration of associating polymer (for the same polymer) is a key factor in determining the effective degree of cross-linking in a reversible gel (*18*). In general, our SR procedure offers a convenient way to determine the network modulus in gluten gels and also to characterize the sol fraction at the same time (or more precisely shorter time). Thus, one is tempted to speculate that $G(\tau^*)$ is fundamentally related to the actual concentration of associating glutenin proteins in gluten that are part of the reversible network. This important subset of the total aggregating glutenin proteins in gluten can probably only be deduced relatively from rheological data due to gliadin−glutenin interactions in gluten. Much remains to be learned about how individual subunits assemble themselves into network structures, but this unique combination of dynamic viscoelastic data and known chemistry of the cultivars used here has been illuminating in this respect. One thing we can say based on the nonrandomness of our SR patterns between cultivars is that this self-assembly process is cultivar specific.

SR of Glutenin. Although we focused on the LVP of gluten gels above because the functional form of wheat proteins in baking is in fact gluten, we were also interested in determining how addition of gliadin affected the network strength of the parent glutenin gels for different cultivars. We hypothesized based on the gluten gel data discussed above that the stronger cultivars with their higher amount of unextractable glutenin protein would withstand plasticization by the uncross-linked gliadin protein fraction better. In other words, we thought that gliadin would preferentially solubilize unconnected glutenin gel clusters into the sol fraction of gluten. One must keep in mind that chemical extraction and fractionation techniques performed on wheat proteins will not give any information on how the fractions interact in gluten. **Figure 5A** shows the relaxation spectra for all of the glutenin gels tested while **Figure 5B** shows the corresponding SR curves. SR parameters were obtained for the gluten gels as described earlier and are tabulated in **Table 4**.

The SR patterns of glutenin gels can be divided into two main modes with the help of the spectra. The first relaxation process is characterized by a broad, nearly "boxlike" spectrum for the first 200 s for all of the glutenin gels (note the small n values). There is a small, but noticeable, peak or shoulder located at about 20 s for all of the cultivars. The spectra are very similar to the first portion of the elution curves for proteins extracted from mixed bread wheat doughs with and without reducing agent from the work by Meredith and Wren (*28*). They noted that mixing doughs in the presence of small amounts of thiols in the form of sodium sulfite resulted in the appearance of a new peak in the elution curve between the HMW glutenin and

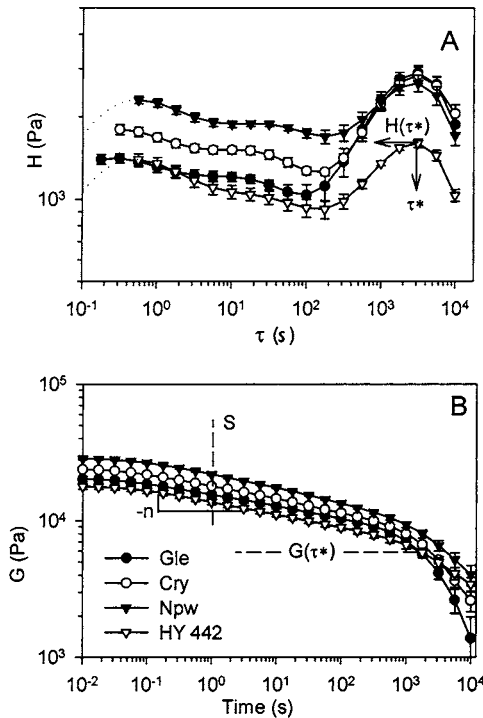

Figure 5. SR spectra (**A**) and the corresponding relaxation modulus (**B**) for four glutenin gels (60% water on total weight basis) obtained from common wheat cultivars of different classes: Gle (CWES), Cry (CPS-R), Npw (CWRS), and HY 442 (CPS-W). The temperature was 25 °C, and the shear strain was 0.5% for all materials.

Table 4. SR Modulus ($G(t)$) and Spectra ($H(\tau)$) Parameters for Glutenin Gels (60% Water on Total Weight Basis) from Various Cultivars Representing Different Canadian Wheat Classes[a]

	$G(t)$ parameters			$H(\tau)$ parameters	
	n	S (Pa s^n)	$G(\tau^*)$ (Pa)	τ^* (s)	$H(\tau^*)$ (Pa)
Gle (CWES)	0.09	15667	4384 ± 531 a	3056 ± 233	2915 ± 189
Cry (CPS-R)	0.10	18167	5168 ± 382 a	3097 ± 205	2861 ± 236
Npw (CWRS)	0.11	22146	6831 ± 475 b	2831 ± 188	2663 ± 184
HY 442 (CPS-W)	0.09	13696	5101 ± 413 c	2845 ± 218	1607 ± 50
Ree (SWS)	0.10	15864	5165 ± 548 c	2158 ± 81	2218 ± 169

[a] Numbers with the same letters in the same column denote a statistically insignificant difference at 95% confidence.

gliadin peaks. They attributed this to the ease of release of a glutenin fragment (or cluster) of MW between 80 000 and 100 000.

The SR spectra patterns for the glutenin gels shown in **Figure 5A** are qualitatively very similar to the patterns of the gel filtration curves for glutenin proteins extracted from defatted wheat cultivars shown in **Figure 1** in Huebner and Wall (*29*). A schematic rendering of the elution pattern for a good quality and poorer quality breadmaking wheat is shown here in **Figure 6**. We think it is important to point out the similarity between the patterns of the glutenin proteins in these gel filtration curves (absorbance vs elution volume) and our relaxation spectra ($H(\tau)$ vs time) for glutenin gels. The mirror image is due to the fact that the largest glutenin clusters elute first but relax last. One can also see in **Figure 6** that the distribution of the gliadin proteins is much narrower than for the glutenin proteins and is more similar for different cultivars than the glutenin proteins. Weaker (poorer) cultivars show smaller peak absorbance in gel chromatography and a smaller peak relaxation intensity in SR.

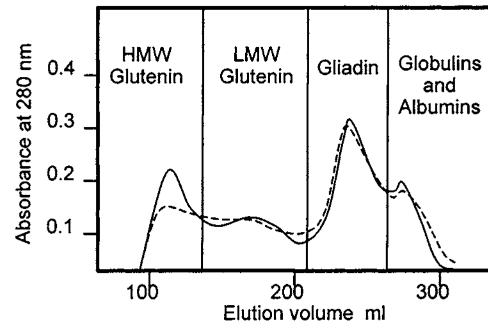

Figure 6. Schematic rendering of gel filtration chromatograms (Sepharose 4B column) for wheat proteins extracted from two wheat cultivars of different bread making quality (Comanche, good quality, solid line; and K14042, poorer quality, dotted line). (Adapted from **Figure 1** of Huebner and Wall, 1976.)

We can also apply the later findings of Graveland et al. (*3*) to surmise that the Glut-1 peak of Huebner and Wall most likely contains SDS insoluble glutenin (as would their insoluble protein fraction as well), while the broader distribution of the Glut-2 fraction represents SDS soluble material. This line of reasoning leads one to speculate that the long time relaxation mode in these glutenin gel spectra represents relaxation processes in the SDS insoluble (cross-linked) gel network, while the earlier and broader relaxation distribution spectra found here could correspond to the relaxation of clusters that are SDS soluble, that is, unconnected to the gel network. This supports our earlier idea that the power law relaxation mode in gluten gels is due to a sol (unconnected glutenin fraction). We are not aware of any previously published work showing this long time relaxation peak in the SR spectrum of glutenin gels or discussing their similarity to gel filtration profiles.

The power law spectra are interesting because the S values for the power law region are in the order Npw > Cry > Reed > Gle > HY 442. For the gluten gels (**Table 2**), the order was ES 12 and Gle > Cry > Npw > HY 442, SWS 231, and Ree. So Npw has the highest S value of the glutenin gels but is intermediate in the gluten gels. The second relaxation region for the glutenin gels consists of a peak in all of the spectra with the maximum intensity for each glutenin gel occurring in a fairly narrow range of relaxation times: 2831 and 2845 s for Npw and HY 442, respectively, and 3056 and 3097 s for the stronger cultivars Gle and Cry, respectively. These maxima in the spectrum correspond to an inflection point in the corresponding glutenin gel relaxation modulus curves, which is the pseudo-plateau modulus for the transient network as discussed above for gluten gels. It is very interesting that the $G(\tau^*)$ values were in the order Npw > Cry, HY 442, and Ree > Gle. For the gluten gels, the order was ES 12 and Gle > Cry > Npw > HY 442 > SWS 231 > Ree. Clearly, the strength (S and $G(\tau^*)$) of gluten gels is in a different order than the glutenin gels. This suggests looking at the ratio of $G(\tau^*)$ for the glutenin and gluten gels as an index of cultivar strength rather than just $G(\tau^*)$ for the glutenin or gluten gels alone. The physical meaning of this ratio would be a relative indication of how the gliadin fraction affects the glutenin gel network strength, a lower number indicating better preservation of the network structure in the presence of gliadin proteins and other main components in the gluten gel.

Comparison of Glutenin and Gluten Gel Relaxation Parameters. Ratios of SR parameters for the glutenin and gluten gels (determined for both at 60% MC) for the same cultivar were calculated and are shown in **Table 5**. The Gle glutenin gel network clearly resists dilution, or dissolution, by the gliadin

Table 5. Ratios (Glutenin Gel/Gluten Gel) of the Power Law Index (n), Power Law Strength (S), and Network Strength ($G(\tau^*)$) for Common Wheats Representing Different Canadian Wheat Classes[a]

	ratio of n for glutenin gel to that of gluten gel	ratio of S for glutenin gel to that of gluten gel	ratio of $G(\tau^*)$ for glutenin gel to that of gluten gel
Gle (CWES)	0.6	7.4	5.6
Cry (CPS-R)	0.5	10.7	9.6
Npw (CWRS)	0.5	24.4	28.2
HY 442 (CPS-W)	0.3	20.6	35.2
Ree (SWS)	0.3	24.5	51.1

[a] Gels were 60% water content on a total weight basis.

fraction much better than weaker cultivars. The differences in the values of the ratio of glutenin $G(\tau^*)$ to that of gluten $G(\tau^*)$ are striking considering all gels were evaluated at the same moisture content and the range of reported gliadin to glutenin ratios ranges from about 0.5 to 0.75. However, the ratios of $G(\tau^*)$ for glutenin and gluten ranged from 5.6 for Gle (extra strong) to 51.1 for Reed (weak), indicating a wide variation in the relative effect of the gliadin fraction on the glutenin network strength. This ratio scale provides an expanded range of cultivar strength or quality, much broader than mixograph mixing time. There is also plenty of room on the scale to differentiate cultivars of the same wheat class but with subtle differences in their quality. This is not seen with the other ratios; for example, the range for n is quite small and not a very good indicator of cultivar strength. The trend of the ratios of S values does not trend with mixing strength. According to the ratio of network strength values, the CPS-R cultivar Cry is much closer to Gle than the CPS-W cultivar HY 442. CPS-R cultivars are recommended for hearth breads such as French breads, which is consistent with their relatively low value of network strength ratio. The ratio of glutenin to gluten network strength also seems to place the various cultivars into separate classes unlike $G(\tau^*)$ for gluten gels alone. Thus, a combination of the current Canadian wheat classes and the ratio of network strengths for glutenin and gluten could provide more complete information on the quality and strength of wheat cultivars to aid in the marketing of cultivars for specific uses.

Tensile Testing of Gluten Gels. The flow properties of gluten under macroscopic deformation represent an important functional property necessary for dough mixing, sheeting, proofing, and oven rise. Thus, it was of interest to determine which, if any, of the linear viscoelastic properties determined here would be good predictors of the tensile stress build-up of gluten gels. Therefore, a crosshead speed of 0.1 mm/s was chosen to match the approximate rate of initial deformation of a bread dough ($\dot{\epsilon} = 2.0 \times 10^{-3}\ s^{-1}$) for the initial sample length used here and presumably the gluten network within the dough, during fermentation and oven rise (*30*). It is reasonable to assume that differences in the tensile stress build-up results reflect relative differences in the rate at which the gluten reversible network dissipates stress internally, presumably by rearrangement of its network structure. It is also reasonable to assume that the gluten in dough is hydrated to a similar degree as these gluten gels, although the volume fraction of gluten protein is much less in dough than in the gluten gels tested here.

Figure 7 shows the tensile stress vs time curves for several glutens. All six gluten samples could be stretched to four times their original length, without fracture highlighting their extensibility, or flowability, under stress. Gle gluten gel exhibited the highest tensile stress build-up. Cry and Npw showed very similar stress responses and were intermediate between Gle and HY 442 and SWS 231 glutens, which showed little or no build-up of stress. In other words, under these particular test conditions, they were essentially viscous liquids.

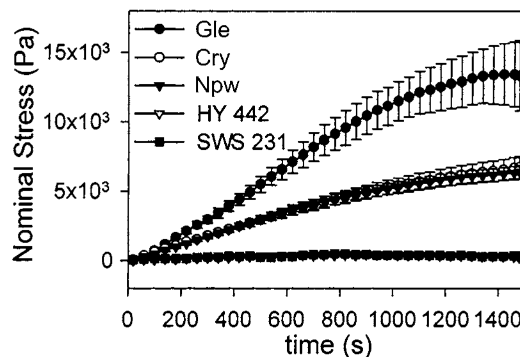

Figure 7. Tensile properties of gluten gels (60% water on total weight basis) from common wheat cultivars representing different classes of Canadian wheat: Gle (CWES), Cry (CPS-R), Npw (CWRS), HY 442 (CPS-W), and SWS 231 (SWS). Crosshead speed was 0.1 mm/s, relative humidity was 94%, and temperature was 25 °C.

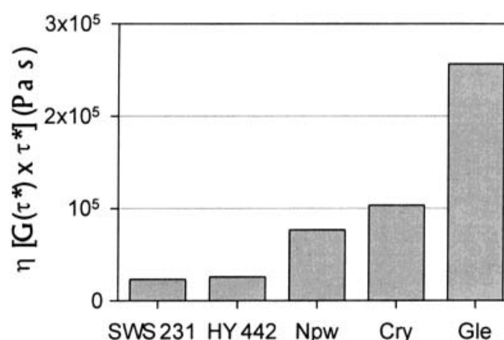

Figure 8. Viscosity at 25 °C of reversible gluten gels at 60% moisture content total weight basis (estimated from $G(\tau^*) \times \tau^*$) for five Canadian wheat cultivars of different classes: Gle (CWES), Cry (CPS-R), Npw (CWRS), HY 442 (CPS-W), and SWS 231 (SWS).

It is interesting that again the stress curve for Npw is intermediate between the Gle and the weaker cultivars, as was also found for the SR patterns for the glutens. However, the tensile stress build-up patterns did not trend that well with the ratio of network strengths for glutenin and gluten as reported in **Table 5**. Npw and Cry have different values of this ratio but the same tensile stress build-up patterns, likewise for HY 442 and SWS 231. The viscosity of the reversible network, which reflects the rate of breaking and reforming of cross-links, may be a better predictor of tensile properties. This viscosity can be estimated from SR data as $\eta_{net} \sim G(\tau^*) \cdot \tau^*$ (*18*). This means that there is a superposition between long relaxation times and network strength, in terms of the effective viscosity of the reversible network. Tensile stress build-up may reflect this network viscosity and not just $G(\tau^*)$. The gluten gel network viscosities were calculated from the relaxation data in **Table 2** and are shown in bar graph form in **Figure 8**. Visual comparison between **Figures 7** and **8** shows that the tensile stress patterns of gluten gels trend much better with the gluten gel network viscosity. Thus, there is a strong linkage between the dynamic viscoelastic properties of gluten gels in the form of their network viscosity and their large deformation flow properties. This result makes sense, since both are in fact dynamic processes, and suggests that the concept of strength alone may be overemphasized as a measure of cereal quality. Thus, a fairly complete characterization of a wheat cultivar would need to include

conventional measures of wheat quality used to establish grades for marketing plus the network viscosity of the hydrated gluten proteins as shown here, as well as dough rheological properties. This may seem excessive, but one could view determination of the dynamic viscoelastic properties of glutenin and gluten gels and doughs as the effect of concentration of glutenin associating polymer at only three different levels.

CONCLUSIONS

In summary, the fundamental dynamic viscoelastic properties reported here support the view that gluten gels are best described as reversible gels near the gel point and contain a sol and gel network fraction. The strength of the gluten gel reversible network ($G(\tau^*)$) increased as the proportion of unextractable glutenin protein increased. A new concept, the ratio of the network strengths for glutenin and gluten gels, was introduced as a fundamental approach to ascertaining the quality of hydrated cereal proteins for a given cultivar. This ratio reflects interactions between the gliadin and the glutenin gel networks, with lower values indicating greater strength, defined as a higher retention of the glutenin gel network strength in the presence of the gliadin fraction. It differentiated very well a group of common wheat cultivars that could not be separated based on HMW subunit composition, ratio of gliadin to glutenin, or physical dough properties alone. However, the tensile stress build up at a low rate of deformation only trended well with the gluten reversible network viscosity and not the network strength alone. The results here suggest that a critical value of the gluten network viscosity must be exceeded before stress build-up is observed at low rates of tensile deformation. This could be important for obtaining good loaf volume as the HY and SWS cultivars have been found previously to give low loaf volumes as compared to Npw and Cry (*9*), which also showed tensile stress build-up here.

On the other hand, HY 442 had a similar viscosity and similar elongational flow curve as SWS 231 but had a loaf volume similar to Npw and other good bread-making cultivars. Thus, there are still mysteries to be unraveled. We are now determining the effect of temperature up to 40 °C and the addition of oxidizing and reducing agents on the dynamic viscoelastic properties of gluten gels for some of these same cultivars. Temperature is the other main factor, in addition to concentration, that affects the dynamics of reversible networks. In any case, the gluten gel network strength and network viscosity can be determined directly from SR experiments but only if the time of the experiment is longer than the longest relaxation time of the gluten gel. This is not the general practice.

ACKNOWLEDGMENT

We gratefully acknowledge our collaborators in this line of research, Dr. J. E. Dexter and Dr. N. M. Edwards, of the Canadian Grain Laboratory (Canadian Grain Commission, Winnipeg, MB) for providing us with the wheat flours used here and determination of their chemical mixing and physical dough properties as cited here (*8*−*10*).

LITERATURE CITED

(1) Nijenhuis, K. T. Calculation of network parameters in thermoreversible gels. *Polym. Gels Networks* **1996**, *4*, 415−433.

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

(3) Graveland, A.; Bosveld, P.; Lichtendonk, W. J.; Marseille, J. P.; Moonen, J. H. E.; Scheepstra, A. A model for the molecular structure of the glutenins from wheat flour. *J. Cereal Sci*. **1985**, *3*, 1−16.

(4) Winter, H. H.; Chambon, F. Analysis of linear viscoelasticity of a cross-linking polymer at the gel point. *J. Rheol*. **1986**, *30*, 367−382.

(5) Rubinstein, M.; Dobrynin, A. V. Associations leading to formation of reversible networks and gels. *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 83−87.

(6) Tanaka, F.; Edwards, S. F. Viscoelastic properties of physically cross-linked networks Part 1. Nonlinear stationary viscoelasticity. *J. Non-Newtonian Fluid Mech.* **1992**, *43*, 247−271.

(7) Leibler, L., Rubinstein, M.; Colby, R. H. Dynamics of reversible networks. *Macromolecules* **1991**, *24*, 4701−4207.

(8) Rao, V. K.; Mulvaney, S. J.; Dexter, J. E.; Edwards, N. M.; Peressini, D. Stress-Relaxation properties of mixograph semolina-water doughs from durum wheat cultivars of variable strength in relation to mixing characteristics, bread- and pasta-making performance. *J. Cereal Sci*. **2001**, *34*, 215−232.

(9) Rao, V. K.; Mulvaney, S. J.; Dexter, J. E. Rheological characterization of long- and short- mixing flours based on stress-relaxation. *J. Cereal Sci.* **2000**, *31*, 159−171.

(10) Edwards, N. M.; Peressini, D.; Dexter, J. E.; Mulvaney, S. J. Viscoelastic properties of durum wheat and common wheat dough of different strengths. *Rheol. Acta* **2001**, *40*, 142−153.

(11) Ferry, J. D. Molecular theory for undiluted amorphous polymers and concentrated solutions; networks and entanglements. *Viscoelastic Properties of Polymers*; John Wiley & Sons: New York, 1980; Chapter 10, pp 224−264.

(12) American Association of Cereal Chemists. *Approved Methods*; American Association of Cereal Chemists: St. Paul, Minnesota, 1983.

(13) Alfrey, T., Jr. *Mechanical Behavior of High Polymers*; Interscience Publisher: New York, New York, 1948.

(14) Cunningham, J. R.; Hlynka, I. Relaxation time spectrum of dough and the influence of temperature, rest, and water content. *J. Appl. Phys.* **1954**, *25*, 1075−1081.

(15) Sprinthall, R. C. The hypothesis of difference. *Basic Statistical Analysis*, 3rd ed.; Prentice Hall: Englewood Cliffs, New Jersey, 1990; Chapter 9, pp 164−192.

(16) Marin, G.; Montfort, J. P. Molecular rheology and linear viscoelasticity. In *Rheology for Polymer Melt Processing*; Piau, J.-M., Agassant, J.-F., Eds.; Elsevier Science B.V.: Amsterdam, The Netherlands, 1996.

(17) Bohlin, L.; Carlson, T. L.-G. Shear Stress Relaxation of wheat flour dough and gluten. *Colloids Surf.* **1981**, *2*, 59−69.

(18) Rubinstein, M.; Semenov, A. N. Thermoreversible gelation in solutions of associating polymers 2. Linear dynamics. *Macromolecules* **1998**, *31*, 1386−1397.

(19) Mours, M.; Winter, H. H. Relaxation Patterns of Nearly Critical Gels. *Macromolecules* **1996**, *29*, 7221−7229.

(20) Rodd, A. B.; Dunstan, D. E.; Ross-Murphy, S. B.; Boger, D. V. Dependence of linear viscoelastic critical strain and stress values on extent of gelation for a thermally activated gelling system. *Rheol. Acta* **2001**, *40*, 23−29.

(21) Winter, H. H. Polymer Gels, Materials that combine liquid and solid properties. *MRS Bull.* **1991**, *16*, 24−26.

(22) Vasil'ev, V. G.; Rogovina, L. Z.; Slonimskii, G. L.; Papkov, V. S.; Shchogelikhina, O. I.; Zhdanov, A. A. The Rheology of Carboxyl-Containing Polydimethylcarbosiloxanes and the Nature of Reversible Physical Network. *Polym. Sci. Ser. A* (*U.S.S.R.*) **1995**, *37* (2), 174−178.

(23) Schofield, J. D.; Bottomley, R. C.; Timms, M. F.; Booth, M. R. The Effect of Heat on Wheat Gluten and the Involvement of Sulfhydryl-Disulfide Interchange Reactions. *J. Cereal Sci.* **1983**, *1*, 241−253.

(24) Plazek, D. J.; Frund, Z. N. Recoverable creep compliance properties of associative model polymer and polyoxyethylene solutions. *J. Rheol*. **2000**, *44*, 929−946.

(25) Jauregui, B.; Munoz, M. E.; Santamaria, A. Rheology of hydroxyethylated starch aqueous systems. Analysis of gel formation. *Int. J. Biol. Macromol.* **1995**, *17*, 49−54.
(26) Lukow, O. M.; Payne, P. I.; Tkachuk, R. The HMW glutenin subunit composition of Canadian wheat cultivars and their association with bread-making quality. *J. Sci. Food Agric*. **1989**, *46*, 451−460.
(27) Flory, P. L. Molecular weight distributions in nonlinear polymers and the theory of gelation. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, New York, 1953; Chapter IX, pp 347−398.
(28) Meredith, O. B.; Wren, J. J. Determination of molecular weight distribution in wheat-flour proteins by extraction and gel filtration in a dissociating medium. *Cereal Chem*. **1966**, *43*, 169−187.
(29) Huebner, F. R.; Wall, J. S. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. *Cereal Chem.* **1976**, *53*, 258−269.
(30) Bloksma, A. H. Rheology of breadmaking process. *Cereal Foods World* **1990**, *35*, 228−236.

Received for review June 28, 2002. Revised manuscript received January 7, 2003. Accepted January 22, 2003. We acknowledge the financial support received for this work from the USDA NRICGP Grant No. 1999-01680.

JF020719L