

Effect of L-Cysteine on the Rheological Properties of Wheat Flour

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ABSTRACT

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Extrudate expansion of cereal-based products is largely dependent on the molecular interactions and structural transformations that proteins undergo during extrusion processing. Such changes strongly influence the characteristic rheological properties of the melt. It is possible to modify rheological properties of wheat flour during extrusion processing, in particular shear viscosity, with cysteine. The objective of this work was to further develop an understanding of the molecular interactions and structural transformations of wheat flour from dynamic oscillatory rheological measurements. Temperature and frequency sweeps were conducted in the linear viscoelastic range of the material. Changes in the storage modulus (G'), the loss modulus (G'') and the loss tangent ($\tan \delta$) of 25%

moisture wheat flour disks as a function of cysteine concentration (0–0.75%) were monitored. Molecular weight between cross-links (M_c) and the number of cross-links (N_c) per glutenin molecule were determined from frequency sweep data. Increasing cysteine concentration broke cross-links by decreasing G' maximum and increasing $\tan \delta$ values. Molecular weight between cross-links increased and the number of cross-links decreased. G' values from temperature sweeps showed a similar trend. This information leads to a better understanding of the viscoelastic behavior of wheat flour doughs during extrusion cooking and elucidation of protein-protein reaction mechanisms and other interactions in extruded cereal-based snack foods.

L-Cysteine and similar thiol compounds play an important role in the modification of the rheological properties of foods, especially as reducing agents and free radical scavengers in the processing of cereal-based foods. Studies in extrusion processing have generally focused on the contribution of process variables and ingredients to the properties of the final product. However, the changes taking place at the molecular level are not well understood despite several efforts to study them systematically (Ledward and Mitchell 1988; Stanley 1989; Strauss et al 1992; Wasserman et al 1992; Ledward and Tester 1994; Strecker et al 1995; Koh et al 1996; Li and Lee 1996a,b). An understanding of the changes taking place at the molecular level must be a major objective if systematic design rules to optimize the quality of the final product are to be developed. While chemical techniques are an excellent way to obtain evidence about chemical transformations, the results are often confusing in terms of understanding their effect on physical properties. Rheological properties, which are very sensitive to key chemical transformations, can serve as a bridge to understanding chemical changes. The relationship between structural changes in the biopolymer melt and the resulting rheological properties are important because chemical changes also influence the development of important extrudate characteristics such as pore size, and textural and mechanical properties.

A number of researchers have proposed that both disulfide bond formation and hydrophobic interaction play a key role in protein cross-linking during extrusion processing and thus in the development of textural characteristics of extruded products (Areas 1992; Martinez-Serna and Villota 1992; Strecker et al 1995; Li and Lee 1996a,b). Intermolecular peptide bonds have also been suggested as being responsible for the cross-linking process with disulfide and hydrogen bonds plus hydrophobic interactions playing secondary roles (Simonsky and Stanley 1982; Stanley 1986, 1989). In addition, protein free radicals are produced during extrusion by thermal and mechanical forces, and reactions and rearrangements of these radicals are also believed to be critically involved in protein cross-linking and textural characteristics of extruded wheat flour (Schaich 1992; Strauss et al 1992; Koh et al 1996). It is possible that all these pathways occur simultaneously during extrusion. What is clear is that the mechanisms of protein-protein interactions during extrusion,

which are responsible for final texturization, are rather complex and remain uncertain, and this has been the main limiting factor for use of this process to its full potential (Areas 1992).

Cysteine, an SH-containing amino acid, can act both as a reducing agent and a free radical scavenger during the extrusion processing of wheat flour (Stauffer 1990; Koh et al 1996; Li and Lee 1996b). Theoretically, protein-protein interactions through covalent disulfide bond formation can be disrupted by added cysteine. In all cases, changes in the structure of the wheat proteins and the molecular interactions occurring must be reflected in the rheological properties of the melt.

The use of dynamic mechanical techniques to characterize the molecular motions and relaxation behavior of viscoelastic materials is increasing (Cocero and Kokini 1991; Amemiya and Menjivar 1992; Kalichevsky et al 1992; Kokini et al 1995; Morales-Diaz 1997; Morales-Diaz and Kokini 1998). In fact, dynamic rheological techniques have been applied to a variety of polymer systems, including foods, to determine fundamental mechanical properties. One type of dynamic testing, sinusoidal stress-strain analysis has been a useful tool in examining the fundamental rheological properties of doughs (Faubion et al 1985; Weipert 1990; Kokini et al 1994; Madeka and Kokini 1994). Structure formation or breakdown in macromolecular systems as a result of conformational changes, state transitions, or interactions with other constituents can thus be probed by continuously monitoring the rheological responses of the sample under small strain. Sinusoidal stress-strain testing can thus be used to examine effect of additives on the rheological behavior of doughs during processing.

In small amplitude dynamic rheometry, when a material is deformed sinusoidally at a frequency ω (rad/sec), the shear strain can be expressed as:

$$\gamma(t) = \gamma_0 \sin(\omega t) \quad (1)$$

where γ_0 is the strain amplitude, ω is the angular frequency, and t is the time. The strain rate, or shear rate, will be the first derivative of strain with respect to time. For a linearly elastic material (Hookean solid), the shear stress will be in phase with the imposed shear strain, while for a linearly viscous material (Newtonian liquid), the shear stress will lead the shear strain by exactly 90°. Thus, for a viscoelastic material the phase angle (δ) between the shear stress and shear strain functions varies within 0° and 90°, giving rise to two stress components, one in-phase and the other out-of-phase. The stress function can be written as:

$$\sigma(t) = \gamma_0 [G' \sin(\omega t) + G'' \cos(\omega t)] \quad (2)$$

$$\text{where } G' = \sigma_0 / \gamma_0 \cos \delta \text{ and } G'' = \sigma_0 / \gamma_0 \sin \delta \quad (3)$$

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The in-phase component represents the elastic character of the material (the storage modulus, G'). It is a measure of the energy stored in the material on sinusoidal deformation and recovered per cycle. The out-of-phase component represents the viscous character of the material (the loss modulus, G''). It is a measure of the energy dissipated or lost as heat per cycle of deformation (Biliaderis 1992).

Another parameter useful in characterizing the physical state of a viscoelastic material is the loss tangent or $\tan \delta$. This dimensionless parameter is the ratio of the energy lost to the energy stored for each cycle of deformation ($\tan \delta = G''/G'$). This parameter is more sensitive than G' or G'' in probing changes in the viscoelastic character of a polymer network (Biliaderis 1992).

This study investigated the effect of added cysteine on the rheological properties of wheat flour in terms of protein-protein interactions using dynamic oscillatory rheometry, and the relationship between such interactions and extrudate expansion behavior.

MATERIALS AND METHODS

Sample Preparation for Pressure Rheometry Measurements

Distilled water was added to the wheat flour in a 100-g resistograph (Brabender Instruments, South Hackensack, NJ) at 63 rpm and 30°C so that a cohesive dough was obtained. Where L-cysteine was used, the relevant additive concentration (0.25, 0.5, and 0.75%) was dissolved in the water before dough preparation. Sodium benzoate (0.05% w/w of the equilibrated dough) was added to the dough to prevent molding of the samples. The dough was then rolled to a height of 2.5 mm from which disks of 20.0 ± 0.2 mm were cut. The samples were equilibrated to a moisture content of $25.0 \pm 0.4\%$ by exposure to a potassium nitrate (KNO_3) saturated salt solution with a water activity of 0.93. The time to reach

equilibrium was determined by weighing equilibrating samples over time until a negligible change in weight was obtained.

Pressure Rheometry Studies

The RPR (Rheometrics Pressure Rheometer, Piscataway, NJ) with parallel plate geometry was used to observe temperature-induced transitions of the material by monitoring changes in G' and G'' . For materials in which water is one of the components and transition temperatures are $>100^\circ\text{C}$, rheological experiments cannot be conducted at atmospheric pressure because water boils at 100°C . In this case, a positive pressure above the vapor of water at the measurement temperature in the headspace of the measurement fixture is necessary (Kokini et al 1994). Wheat flour dough samples were pressed into disks 2.2 mm in height and 20 mm in diameter. Strain sweeps of 0.5–100% showed the linear viscoelastic region of the samples to be 0.5–0.8% at a frequency of 0.75 rad/sec. Temperature dependence of G' and G'' was determined at a heating rate of $5^\circ\text{C}/\text{min}$, a strain of 0.7%, and frequency of 0.75 rad/sec over a temperature range of $40\text{--}160^\circ\text{C}$.

Frequency sweeps within 0.1–100 rad/sec, 0.7% strain, and 200 psi pressure were conducted at 140°C to determine the effect of additive concentration on the storage modulus (G'), the loss modulus (G''), and the loss tangent ($\tan \delta$) of the wheat flour. This temperature simulated earlier on-line rheological measurements during extrusion processing. All experiments were done in duplicate.

Determination of Molecular Weight Between Cross-Links (M_c)

The effect of additive concentration on the extent of cross-linking in wheat flour was determined by calculating the average molecular weight between cross-links (M_c) from the plateau modulus G' value. M_c calculation (Muller 1969) used the equation:

$$M_c = (\rho RT)/G' \quad (4)$$

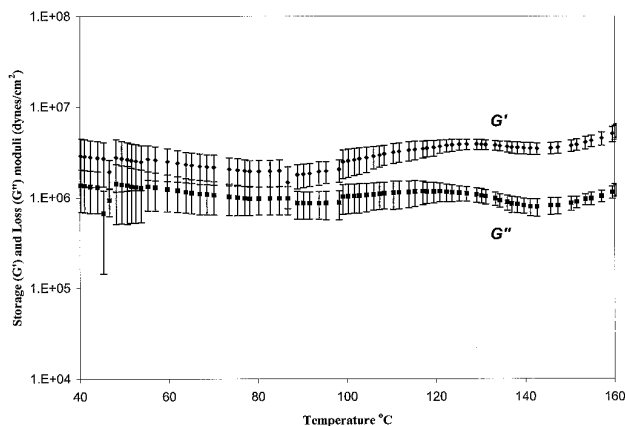


Fig. 1. Average temperature sweep: wheat flour (25% mc), 0% additive.

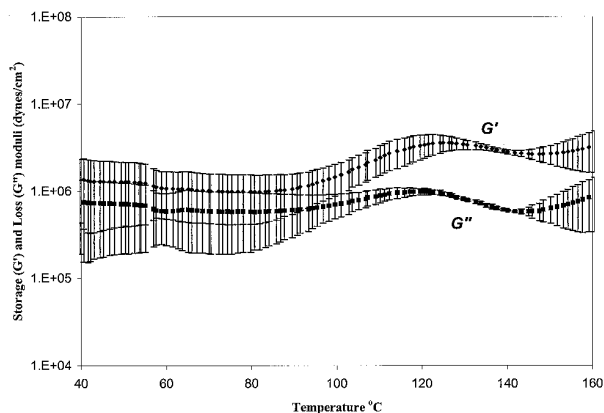


Fig. 2. Average temperature sweep: wheat flour (25% mc) + 0.25% cys.

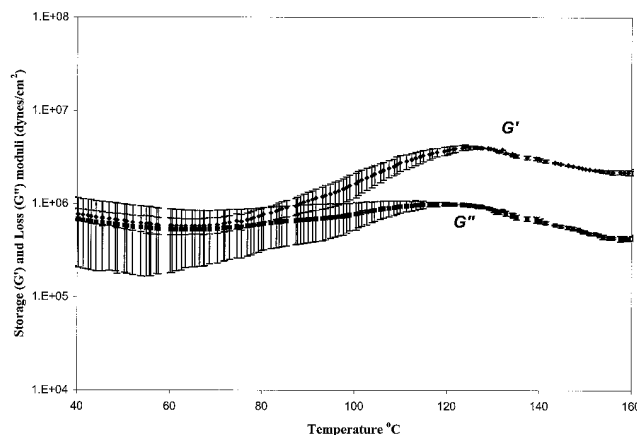


Fig. 3. Average temperature sweep: wheat flour (25% mc) + 0.5% cys.

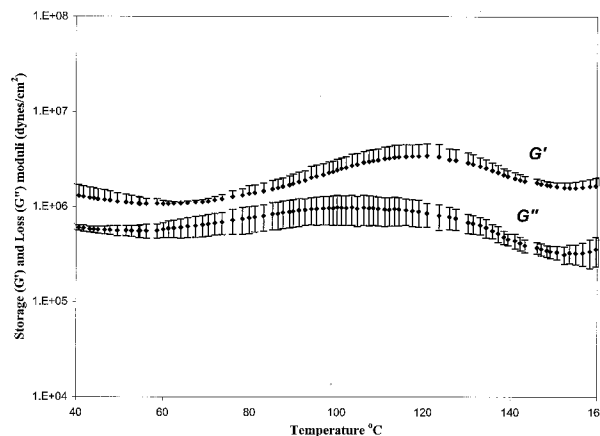


Fig. 4. Average temperature sweep: wheat flour (25% mc) + 0.75% cys.

where ρ is the material density, R is the gas constant, T is the absolute temperature, and G' is the storage modulus of the cross-linked material. From the plateau modulus (maximum G' value obtained from the frequency sweep), the average molecular weight between cross-links as well as the number of cross-links per average molecule (N_c) were calculated:

$$N_c = \text{Molecular weight (MW)} / 2 M_c \quad (5)$$

where an average molecular weight of 100,000 was used for the glutenin subunit. The average density of the wheat flour disks was 1.4 g/cm³. The average density was determined by dividing the average mass of 10 samples by the average cylindrical volume.

RESULTS AND DISCUSSION

Temperature Sweeps

A typical graph of the change in storage (G') and loss (G'') moduli with respect to temperature for wheat flour at 25% moisture, without additive, at a frequency of 0.75 rad/sec, a strain of 0.7%, and heating rate of 5°C/min is shown in Fig. 1.

At 40 ± 1°C, the average storage modulus (G') was 2.9 ± 1.57 × 10⁶ dynes/cm² and the loss modulus (G'') 1.37 ± 0.68 × 10⁶ dynes/cm². This magnitude of G' suggests a lightly cross-linked structure in the structure flow region. The magnitude of both G' and G'' gradually decreased as temperature increased from 40°C. These values reached a minimum of 1.47 ± 0.72 × 10⁶ and 9.8 ± 3.3 × 10⁵ dynes/cm², respectively, at ≈87°C. This initial decrease in G' is generally associated with entangled polymer flow behavior with increased molecular motion, and thus greater mobility.

As the temperature increased to >90°C, a gradual increase in G' was observed. This can be attributed to cross-linking reactions occurring among the gluten proteins resulting in the formation of a network structure. As suggested in the literature (Booth et al 1980; Schofield et al 1983; Kokini et al 1994), protein-protein interactions through thiol-disulfide interchange reactions would begin to provide an increasingly highly cross-linked structure resulting in higher G' and generally lower G'' values.

Dreese et al (1988a) found that during temperature scans of flour-water doughs at 25–90°C, G' values decreased slowly as the dough temperature increased from 25 to 50°C. At ≈55°C, G' began to increase rapidly reaching a peak at ≈75°C. Starch gelatinization, gluten cross-linking, or both, were advanced as possible explanations for the thermally induced rheological change occurring between 55 and 75°C. However in gluten-starch doughs, the increase in G' during heating from 55 to 75°C was proportional to the starch content of the dough. Thus, they attributed rheological changes occurring as the dough was heated from 55 to 75°C to changes in the starch fraction, presumably because of starch gelatinization.

Using pressure rheometry, Cocero (1993) observed that for glutenin at 11% moisture content, G' decreased with increasing temperature up to ≈90°C. Above this temperature, glutenin entered the

reaction zone, reaching maximum structure build-up at 3.4 × 10⁶ to 6.1 × 10⁶ dynes/cm² at 122–135°C. At 150°C, softening began to occur. Similar thermal transitions were observed for glutenin at 20, 30, and 40% moisture, with the reaction zone beginning at 65°C, maximum structure build-up occurring at 122°C, and softening at 145°C. These transitions did not follow the behavior expected from starch (Attenburrow et al 1990) and were clearly associated with gluten transitions.

Madeka (1996) studied the changes in storage (G') and loss (G'') moduli with respect to temperature for gliadin at 25% moisture. In that case, both G' and G'' reached a minimum at 70°C, followed by a large increase in G' above that temperature. G' reached a peak value of 3 × 10⁶ dynes/cm² at 120°C, with softening occurring at 135°C.

In this work, for wheat flour at 25% moisture content, the storage modulus (G') reached an average maximum value of 3.9 × 10⁶ dynes/cm² at 122–132°C. The average loss modulus (G'') was 1.1 × 10⁶ dynes/cm². At 140°C, the storage (G') and loss (G'') moduli reached average values of 3.5 × 10⁶ and 8.2 × 10⁵ dynes/cm², respectively. While these results are generally consistent with those of both Cocero (1993) and Madeka (1996), in their work these transformations were due to protein-protein interactions. In this work, the situation is complicated by the presence of starch.

Results of temperature sweeps of the other wheat flour samples with 25% moisture content and additive concentrations of 0.25, 0.5, and 0.75% conducted at the same frequency, % strain, and heating rate showed similar patterns (Figs. 2–4, Table I). The effect of additive concentration on both G' and G'' at 140°C are shown in Fig. 5.

At 40°C, samples with added cysteine showed a general decrease in initial G' values. This suggests that, even during mixing, the viscoelastic behavior of the dough is affected by depolymerization caused by cysteine, resulting in a decrease of initial G' values. As temperature increases to 40–90°C, the temperature at which G' minimum occurs decreases. This is consistent with the wide temperature range over which starch gelatinization occurs in wheat flour (Blanshard 1979), and also suggests that the temperature at which cross-linking reactions may occur is also shifted downward due to increased mobility of depolymerized molecules. Thus, starch gelatinization would tend to mask any protein-protein interactions occurring in that temperature range.

On the basis of this work and those of several other researchers (Eliasson and Hegg 1980; Schofield et al 1983; Dreese et al 1988a,b; Attenburrow et al 1990; Cocero 1993; Madeka 1996), it can be concluded that, while increases in G' at 60–90°C may be attributed to starch gelatinization, it is unlikely to be responsible for the increase in G' at >90°C. Above this temperature, protein cross-linking occurs, as shown by a large decrease in protein extractability by SDS (Schofield et al 1983). However, it is important to note that the general shape of the temperature sweep is possibly due to starch gelatinization (Kokini et al 1992). The effect of the protein

TABLE I
Average G' Values (dynes/cm²) of Temperature Sweeps (40–160°C) for Wheat Flour Samples with Cysteine

Cysteine Conc. (% w/w)	G' at 40 ± 1°C	G' Minimum	G' Maximum	G' at 140 ± 1°C
0	29 ± 1.6 × 10 ⁶	1.75 ± 1 × 10 ⁶ at 77–82°C	4 ± 1 × 10 ⁶ at 122–132°C	3.5 ± 1 × 10 ⁶
0.25	1.8 ± 1 × 10 ⁶	1.23 ± 1 × 10 ⁶ at 51–76°C	3.9 ± 1 × 10 ⁶ at 115–125°C	2.07 ± 1 × 10 ⁶
0.5	1.32 ± 1 × 10 ⁶	1.4 ± 1 × 10 ⁶ at 73–90°C	1.95 ± 1 × 10 ⁶ at 120–130°C	1.53 ± 1 × 10 ⁶
0.75	1.39 ± 1 × 10 ⁶	8.01 ± 1 × 10 ⁵ at 77°C	1.18 ± 1 × 10 ⁵ at 121°C	1.01 ± 1 × 10 ⁶

TABLE II
Results of Frequency Sweeps for Wheat Flour with Cysteine (140°C, 25% mc)

Cysteine Conc. (% w/w)	Average G' (dynes/cm ²)	Average G'' (dynes/cm ²)	Tan δ	Slope	M_c (g/mol)	N_c
0	4.02 ± 0.32 × 10 ⁶	3.07 ± 0.54 × 10 ⁵	0.07	0.07	11,962	4.17
0.25	3.01 ± 0.59 × 10 ⁶	3.61 ± 1.26 × 10 ⁵	0.12	0.07	15,976	3.13
0.5	2.19 ± 0.2 × 10 ⁶	2.91 ± 0.98 × 10 ⁵	0.13	0.13	21,958	2.28
0.75	1.96 ± 0.13 × 10 ⁶	3.28 ± 0.318 × 10 ⁵	0.17	0.11	24,535	2.04

network is more clearly observed through the action of cysteine on the magnitudes of G' and G'' at 140°C.

In the reaction zone, G' values reached a maximum at 104–130°C, with G' values relatively constant. This could be due to the masking effects of starch. However, the effect is more clearly observed at 140°C, the temperature that simulates that used for on-line rheological measurements during extrusion, where dramatic decreases in G' values are seen. Figure 5 shows that while cysteine effectively decreased G' values at all concentration levels, decreases in the G'' values were not as dramatic. This suggests that cysteine may be breaking intermolecular disulfide bonds in the gluten polymer network or may be preventing their formation. This is observed in lower G' values reflecting a loss of elastic character in the gluten network.

Frequency Sweeps

Frequency sweeps of wheat flour with 25% moisture and varying levels of additive concentration (0–0.75%) were conducted at a strain rate of 0.7% and temperature of 140°C. This temperature was used in an attempt to obtain data that would mimic the data obtained from on-line rheological measurements. In addition, this temperature also lies in the reaction zone of glutenin and gliadin as shown by Cocero (1993) and Madeka (1996). Sweeps at each additive concentration were conducted in triplicate, and average G' and G'' values were obtained. $\tan \delta$ values were then calculated from the average G' and G'' values. Average results of these sweeps with standard deviations are shown in Table II and Fig. 6. The average molecular weight between cross-links (M_c) and the number of cross-links per average molecule (N_c) are also shown in Table II.

The changes in the storage modulus (G'), the loss modulus (G'') and loss tangent ($\tan \delta$) with increasing cysteine concentration during the frequency sweeps were consistent with the molecular and structural transformations suggested from previous on-line rheological studies and the temperature sweeps. Average G' values in the plateau region were 4.02×10^6 , 3.01×10^6 , 2.19×10^6 , and 1.96×10^6 , respectively, with corresponding $\tan \delta$ values of 0.07, 0.12, 0.13, and 0.17. These values show a generally progressive decrease in the storage modulus (G') value, further strengthening the hypothesis that cysteine breaks disulfide cross-links. In addition, because all experiments were conducted at the same temperature, the decrease in the elastomeric behavior of the cross-linked structure must be attributed to the most likely depolymerization of high molecular weight glutenin molecules. Cysteine concentration appeared to have an effect on the relative independence of $\log G'$ vs. $\log \omega$ at higher cysteine concentrations 0.5 and 0.75% with values of 0.13 and 0.11, respectively. This relative dependency at higher concentrations further reflects less elastic character in the material, again due to depolymerization of high molecular weight units.

For cross-linked polymers, the average value of the storage modulus (G_e') in the plateau region is related to the average molecular

weight between cross-links (M_c) by the relationship $G_e = \rho RT/M_c$. From this relationship, the extent of the cross-link reaction experienced by wheat flour with cysteine at varying concentrations can be obtained from the determination of the molecular weight between cross-links (M_c) from the equilibrium storage modulus (G_e'). Thus, for wheat flour without any additive, the molecular weight between cross-links was 11,962 g/mol. For wheat flour at cysteine concentrations of 0.25, 0.50, and 0.75%, these values rose to 15,976, 21,958, and 24,535 g/mol, respectively.

From these frequency sweeps, the number of cross-links per average molecule (N_c) was estimated by assuming molecular weights of 100 kDa for the glutenin molecule subunits that are stabilized by interchain disulfide bonds (Tatham et al 1990). Using the equation:

$$N_c = MW/2M_c \quad (5)$$

the number of cross-links per average molecule were 4.17, 3.13, 2.28, and 2.04 for wheat flour and added cysteine concentrations at 0.25, 0.5, and 0.75%, respectively.

Based on the thiol-disulfide interchange reaction mechanism for cysteine, the overall effect is to reduce the average molecular weight of the proteins through depolymerization or disruption of the cross-linking process. These results are consistent with those of Dreese et al (1988b), who observed a strong response in G' values when commercial gluten-water doughs were treated with cysteine. Berland and Launay (1995) observed a similar effect on wheat flour dough when glutathione was added at levels >50 ppm to the dough. In these cases, a decrease in G' and increase in $\tan \delta$, characteristic of the reduction in cross-linking in a polymer system, was consistent with the generally accepted theory of the cysteine-induced softening mechanism through breaking or inhibiting the formation of disulfide links between gluten forming proteins.

From a mechanistic approach, Berland and Launay (1995) concluded that the sensitivity of rheological properties of dough to reduction suggests that disulfide bonds play a major role in dough rheology. Glutathione, behaving as a reducing agent, is able to cause gluten breakdown, rupturing disulfide cross-links in gluten by SH-SS interchange. High glutathione content modified the balance between viscous and elastic properties, with G' decreasing more rapidly than G'' . The phase angle also increased with glutathione content. Depolymerization reaction pathways for SH-SS interchange would thus be consistent with those suggested by previous researchers (Koh et al 1996; Li and Lee 1996a,b).

Dong and Hosney (1995) also found that in wheat flour doughs glutathione reduced G' more rapidly than G'' as glutathione content increased from 5 to 150 ppm. They also concluded that the presence of glutathione increased the rate of thiol-disulfide interchange reactions. However, from a mechanistic viewpoint, glutathione was thought to react with low molecular weight sulfhydryl groups and form stable glutathione disulfide (GS-SG). The authors concluded that this can

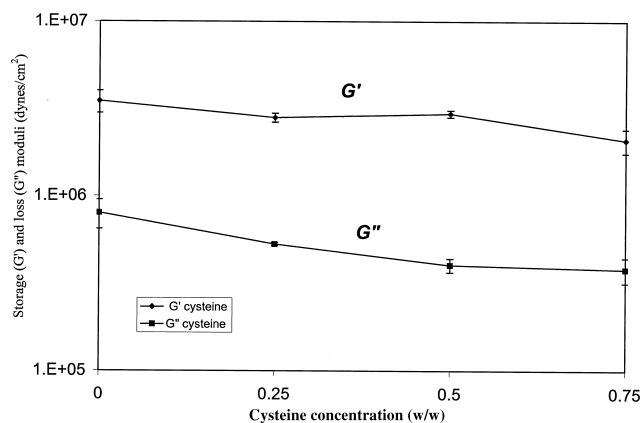


Fig. 5. Effect of cysteine on storage (G') and loss (G'') moduli at 140°C extrusion simulation temperature (25% mc).

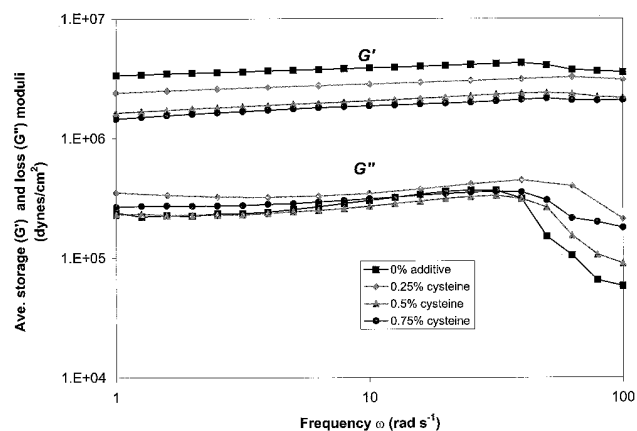


Fig. 6. Comparison of frequency sweep for wheat flour (25% mc) storage (G') and loss (G'') moduli with and without cysteine at 140°C.

be viewed as free radical scavenging, consistent with one of the possible reaction pathways of cysteine with wheat flour, radical scavenging, or quenching by hydrogen donation suggested by Koh et al (1996).

CONCLUSIONS

Results obtained from temperature and frequency sweeps of wheat flour with added cysteine clearly show that cysteine breaks the formation of disulfide cross-links when added to wheat flour during processing. As a result molecular weight between cross-links increased, and the number of cross-links per average molecule decreased. Possible reaction pathways have been advanced by Koh et al (1996), Li and Lee (1996a,b), and Strecker et al (1995).

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