GLUTEN AND THE STARCH-GLUTEN INTERFACE

IN RELATION TO THE

VISCOELASTIC PROPERTIES OF DURUM DOUGH

BY

Nancy Marjorie Edwards

A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements
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DOCTOR OF PHILOSOPHY

Food and Nutritional Sciences
University of Manitoba
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FACULTY OF GRADUATE STUDIES

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ABSTRACT

Processing properties and behaviour of dough under various processing conditions may be predicted through an understanding of dough fundamental rheological properties. A series of studies were conducted, initially to develop small-scale methods that would discriminate between samples varying in dough strength, followed by enrichment studies to assess the contributions of gluten and its fractionated components, and lastly to examine the role of the gluten-starch granule interface in dough viscoelastic behaviour. Dynamic and creep compliance measurements were made in the linear viscoelastic regime, where response was independent of applied stress or strain. An experimental protocol for large deformation creep measurements was found to predict dough extensibility well.

Effects of dough moisture content, protein content and wheat growing environment on dynamic and shear extensibility measurements were assessed. Dough moisture content was shown to affect both tests. However, dough samples always ranked in the same order at a given water absorption level. Subsequent testing of doughs was conducted at fixed absorption. Protein content did not have a significant effect ($p>0.05$) on storage modulus (G'), but did strongly affect tan δ (G"/G'). Tan δ and shear extensibility both increased with increasing protein content.

In addition to samples varying in protein content, a series of durum cultivars ranging in intrinsic dough strength was analyzed. Dynamic measurements proved capable of

discriminating between durum samples ranging in dough strength. Mechanical properties were strongly correlated with many of the alveograph and micro-mixograph parameters. Tan δ was again strongly influenced by protein content.

Recognizing that protein content affected dynamic measurements, creep compliance tests were conducted using a series of common wheat and durum wheat dough samples with a relatively narrow range in protein content. A creep time of 10,000 s was sufficient to reach steady state flow for all doughs. Creep compliance curves were modeled using a six parameter Burgers model, and interpreted in the context of physical gels with reversible crosslinks and entanglements. The entire elastic compliance curve shifted to lower values and steady state viscosity increased as the strength of durum dough (as measured by extensigraph) increased. The elastic compliance curves for common wheat doughs were steeper and the steady state viscosities were lower than for durum doughs of comparable strength.

Retardation spectra offered some clues to the source of rheological differences in durum wheat doughs versus common wheat doughs. Differences between wheat classes were clearly evident for longer retardation times. Retardation strength intensity at longer times decreased as dough strength increased for durum doughs, but increased with increased dough strength for common wheat doughs. The lowest intensity peaks were associated with the highest viscosity among the durum doughs, while the highest intensity peaks were associated with the highest viscosity for the common wheat doughs. The behaviour of durum doughs suggested a strong influence of physical

crosslinks on the linear viscoelastic properties, and that durum dough strength was a function of density of crosslinks present. The greater extensigraph extensibility of common wheat doughs compared with durum wheat doughs was attributed to a greater proportion of higher molecular weight material in the glutenin fraction of common wheat. The hypothesis was advanced that the balance between dough strength and extensibility was dependent on the relative distribution of long chains to short chains and their underlying molecular mobilities.

Enrichment studies aimed at isolating the contribution of various gluten components in durum dough were conducted on a constant protein basis in order to minimize any variability that may be attributable to protein content, thus allowing direct comparisons among cultivars. Gluten was isolated from three durum wheat cultivars with a range in strength and further fractionated to yield gliadin, glutenin and high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits (GS). Dough mixing strength increased with addition of gluten. Dynamic and creep compliance responses ranked in order of strength of the gluten source. Gliadin demonstrated its contribution to the viscous nature of dough with increased tan δ in gliadin enriched doughs. Addition of gliadin had essentially no effect on compliance because of the inability of gliadin to form a network structure. Glutenin enrichment increased overall dough strength, with increased mixing strength, increased moduli, reduced tan δ, and lower creep compliance. The source of glutenin influenced dynamic and compliance responses. Addition of both HMW-GS and LMW-GS resulted in increased dough mixing strength. Creep compliance of doughs with added HMW-GS were in all cases lowered, indicating

a positive effect on dough strength, but with essentially no difference due to source. The source of LMW-GS did affect creep compliance response. Enrichment with LMW-GS from the strong and intermediate cultivars (LMW-2 types) reduced compliance equally and to a greater extent than for the weakest cultivar (LMW-1 type). The mechanisms involved were described in terms of reversible physical crosslinks arising from hydrogen bond and disulfide bond density.

The final study in this series assessed the contribution of starch and starch-gluten surface interactions to durum dough linear viscoelastic properties using a gluten-starch model system. Proportions of starch were substituted in dough with an inert filler, glass powder, having a similar particle size range to starch granules. Doughs were subjected to dynamic and creep compliance measurements. Dough linear viscoelastic properties were weakened upon substitution of starch with glass powder up to 50%, inferring a reduction in adhesion between filler and matrix with decreasing proportions of starch. When glass powder exceeded 50% substitution of starch, the bulk rheological properties of the glass powder exerted a greater influence than the filler-matrix interface and the moduli increased. At high volume fractions of glass powder creep compliance curves exhibited behaviour consistent with a more solid-like system. Surface modification of the glass powder by adsorption of bovine serum albumin (BSA) resulted in reduced dynamic moduli and increased creep compliance in comparison with non-coated glass. Heat treatment of starch granules used in gluten-starch dough lead to increased moduli, lower tan δ, and reduced creep compliance, probably through promotion of hydrophobic interactions with hydrated gluten. Masking the hydrophobic surface of heat-treated

starch granules by adsorption of BSA reduced gluten-starch interactions, confirming the importance of starch granule surface properties in determining dough linear viscoelastic properties.

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CHAPTER 1

Introduction

Although durum wheat has been processed for centuries into pasta and bread, there is still incomplete understanding of the mechanisms involved in dough elastic behaviour and its ability to flow and to recover following deformation. As well, we continue to puzzle over relationships between protein composition and dough functionality, and of the role of starch-protein surface interactions in dough functional properties.

Much of the work that has attempted to relate protein structure to dough functionality has been done on common wheats. Durum wheat and common wheat share common ancestry, both possessing A and B genomes, while common wheats all possess an additional set of chromosomes known as the D genome. Common wheats represent the largest wheat production worldwide, and therefore have been afforded the greatest research interest. As a result research aimed at understanding and explaining durum wheat dough properties has been overlooked until relatively recently.

Common wheat is used for bread production, which is a much more complex product than durum pasta. Different types of breads are prepared using a variety of processes and formulations, often requiring different dough properties. Pasta, in comparison, is a relatively simple product, generally comprised of semolina–water dough that is either extruded or sheeted with shapes pressed out, followed by drying.

An important common feature found in both common wheat and durum wheat is gluten, the functional protein that confers upon dough its viscoelastic properties. Dough is formed when semolina/flour is mixed with water. Gluten protein becomes hydrated forming a continuous matrix surrounding embedded starch granules. Hydrated gluten is highly cohesive, exhibiting both elasticity and extensibility (or viscous flow). Gluten protein is composed of gliadin, a single chain polypeptide containing intra-chain disulfide bonds or no disulfide bonds (ω-gliadin), and glutenin, a polydisperse polymer of varying size with inter-chain disulfide bonds linking individual polypeptide subunits. The two fractions differ in their functional properties. Glutenin is considered responsible for the elastic properties of gluten and dough, while gliadin is believed to contribute to viscous flow, or extensibility characteristics. The ratio of gliadin to glutenin affects dough mixing properties and pasta quality, with increasing proportions of glutenin resulting in increased dough strength and improved pasta texture (Autran et al. 1987).

Glutenin polymer, after reduction of inter-chain disulfide bonds, may be further categorized into two groups according to molecular weight as either high molecular weight (HMW) or low molecular weight (LMW) glutenin subunits (GS). Research on glutenin over the past several years has largely focussed on the contribution of HMW-GS to bread-making quality, where HMW-GS are believed to be of greatest importance in determining common wheat dough elasticity (Shewry et al. 1992). The correlation between dough strength and HMW-GS is not as strong in durum wheat as it is in

common wheat (Liu et al. 1996). Durum wheat contains far less HMW-GS than the hexaploid wheats because it lacks the D genome that encodes for a large proportion of HMW-GS found in bread wheats (Shewry et al. 1997).

Attention in durum wheat has been directed at LMW-GS and their role in pasta quality. The presence of LMW-GS designated as LMW-2 has been associated with good quality pasta, while durum wheat containing LMW-1 is generally of poor pasta-making quality (Boggini et al. 1995). There has been some suggestion that because LMW-2 is expressed in greater quantities it is the amount of LMW-GS that affects pasta quality (D'Ovidio et al. 1999). To date, information on the contribution of the various protein fractions to durum dough functional properties has been based primarily on statistical correlation studies that have not necessarily indicated cause-and-effect relationships.

The capability of relating composition and structure of durum wheat proteins to dough functional properties is complicated by several factors. These include the heterogeneity of the various protein fractions, and variations in both absolute and relative quantities of protein fractions in native semolina samples, making direct comparisons between samples untenable. Osborne (1907) was the pioneer of wheat protein fractionation based on solubility of protein classes. Clean separation of the protein classes has been very difficult because of overlapping solubility of proteins that are comprised of what is essentially a highly heterogeneous mixture of polypeptides (Schofield and Booth 1983). Over the past several years methods have been developed that provide cleaner separation of wheat protein fractions, with the additional caveat that fractionation

methods used had minimal adverse affects on functional properties (Fu et al. 1996, Fu and Kovacs 1999, Marchylo et al. 1989). Owing to the work on protein isolation, various protein fractions may now be isolated and their contributions measured directly by addition to a control sample. This was the approach selected for the studies discussed in this thesis.

There has been little effort made to determine the contribution of starch to dough rheological properties, even though it constitutes approximately 75% of the solids in dough (Colonna et al. 1990). Rasper and deMan (1980) and Lelievre and co-workers (1987) looked at the effects of granule size and water binding capacity on dough rheological properties, but came to opposing conclusions. Rasper and deMan (1980) concluded that water-binding capacity of the starch may play more of a role in dough rheological properties than specific surface area of the granules, whereas Lelievre and co-workers (1987) did not see any clear effect of granule size on water absorption. They suggested that the surface area of the granule over which gluten could spread was more important. Larsson and Eliasson (1997) modified starch granule surface properties and made comparisons with native starch, concluding that interactions between starch granule surface and gluten can influence rheological behaviour of dough.

Before examining the role of specific protein fractions and gluten-starch surface interactions, it was necessary to develop methods that differentiated among dough samples of varying strength while providing information on the possible mechanisms that formed the basis of those differences. The approach chosen involved the use of

traditional empirical measurements (mixograph, farinograph, alveograph, and extensigraph) to provide information on dough strength and extensibility that would be commonly used by processors. In addition, methodologies that used small-scale extensibility measurements and rheological measurements taken in the linear viscoelastic regime (where results are independent of applied stress or strain) were developed to provide information on the fundamental material properties. Once methods were established that could reliably demonstrate differences in fundamental parameters that were related to traditional measurements of dough strength, hypotheses were developed as to the mechanisms involved. Hypotheses were applied in conducting enrichment studies using isolated protein fractions to identify the contributions of the various fractions to dough functional properties and to attempt to explain the mechanisms involved. Finally, the role of starch in dough viscoelastic properties was studied using a gluten-starch model system. Understanding of fundamental rheological parameters of semolina dough will provide the capability to objectively predict processing characteristics and to manipulate processes to obtain the desired end product.

CHAPTER 2

Literature Review

Protein Composition and Functional Properties

Protein quantity and quality are recognized as being of primary importance in the production of high quality wheat based products. Gluten protein is the major contributor to rheological properties of dough. Gluten is composed primarily of two component classes of protein: gliadin and glutenin. Gliadin, made up of single chain molecules with a molecular weight range of 30,000 to 80,000, is considered to contribute to the viscous character of gluten (Bushuk 1986). Gliadins either lack disulfide bonds, as in the ω-gliadins, or contain intra-molecular disulfide bonds and are generally accepted as those gluten proteins that are soluble in aqueous alcohols (Shewry et al. 1997). Glutenin proteins are polymeric molecules stabilized by inter-chain disulfide bonds, and as such their functionality is dictated by their structure (MacRitchie 1992). Molecular weight of the intact (unreduced) glutenin polypeptide has been estimated by asymmetrical flow field-flow fractionation to range from 440,000 to 11 million (Wahlund et al. 1996). Glutenins are considered to contribute to the elastic character of gluten (Bushuk 1986).

Reduction of glutenin disulfide bonds results in release of subunits varying in molecular weight that are designated as either high molecular weight (HMW) glutenins or low molecular weight (LMW) glutenins (Shewry et al. 1997). The estimated molecular

weight of HMW glutenin subunits ranges from 60,000 to 120,000 (Anderson et al. 1988), while that of LMW glutenin subunits ranges from 12,000 to 60,000 (Feillet et al. 1989).

A proposed structure for HMW glutenin describes it as having a central region of repeated sequences with repeating β turns resulting in a loose spiral configuration (Parchment et al. 2001, Shewry et al. 1994). This proposed spiral configuration may be intrinsically elastic, explaining the contribution of HMW glutenin to the elastic properties of dough (Tatham et al. 1990). Shewry et al (1999) proposed that HMW glutenin subunits form the "backbone" of glutenin polymers, with the cysteine residues present in the N- and C- terminal domains. Cysteine residues participate in inter-chain disulfide bonds that act to stabilize the glutenin polymer. They also suggested that the repetitive domains of adjacent subunits form inter-chain hydrogen bonds, which may contribute to gluten viscoelasticity via a "train and loop" mechanism.

In common wheats the presence or absence of particular HMW glutenin subunits has been associated with having positive effects on the breadmaking capabilities of wheat flour (Payne 1987, Shewry et al. 1994). Boggini and coworkers (1995) rendered similar conclusions when studying the breadmaking characteristics of durum wheats using lines with novel glutenin subunit compositions. They observed that dough strength characteristics measured by farinograph, mixograph and alveograph, and bread loaf volume were associated with the presence of particular HMW glutenin subunits (2+, 1 or 1*). Quality differences due to allelic variation in expressed HMW glutenin subunits

of common wheats (hexaploids consisting of A, B and D genomes) are particularly associated with chromosome 1D (Shewry et al. 1997). Durum wheat lacks the D genome. The relationship in durum wheats between HMW glutenins and dough strength is less pronounced than in common wheats (Liu et al. 1996), and is in many cases contradictory (Boggini and Pogna 1989, duCros 1987, Ruiz and Carrillo 1995).

The presence of a particular gliadin band, denoted as band γ-45 by its electrophoretic mobility, has been associated with superior pasta making quality in durum wheats, while the presence of gliadin band γ-42 is indicative of poor pasta making quality (Damidaux et al. 1980). Since that initial finding it has been established that gliadin bands γ-42 and γ-45 are markers that are genetically linked to LMW glutenins encoded at the *Glu-B3* locus (Payne et al. 1984). Differences in the LMW glutenins result in functional differences in pasta quality (Autran and Galterio 1989, duCros 1987). LMW glutenins are composed of many individual subunits, as determined by two-step one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) following protein reduction. LMW glutenins found in durum that are associated with gliadin γ-42 are designated as LMW-1, and those associated with gliadin γ-45 as LMW-2 (Payne et al. 1984). The presence of LMW-2 is considered beneficial to pasta cooking quality (Kovacs et al. 1995). Several LMW subunit patterns are common to both LMW-1 and LMW-2. Of those that differ, LMW subunits 1+14, which are linked to γ-45, have significant positive effects on gluten strength, while subunits 6+11 (linked to γ-42) are negatively associated with gluten strength using the SDS sedimentation method (Vázquez et al. 1996). Boggini and coworkers (1995) found that LMW-2 subunits were

the major contributing factor to dough strength measured by alveograph. Another suggested explanation for the different quality attributes between the two LMW-GS types was that LMW-2 proteins were expressed in greater amounts than LMW-1 types and that overall the effect on gluten quality was a mainly quantitative issue (Autran et al 1987, D'Ovidio et al 1999).

The ratio of HMW:LMW material was determined to be an important factor in functional characteristics such as mixograph dough development time, maximum extensibility, and loaf volume in common wheats when using a short fermentation baking procedure (Gupta et al. 1992, MacRitchie 1992). Gupta et al. (1992) also found that 68 to 80% of the variation in dough extensibility, farinograph dough development time, and loaf volume, when using a long fermentation baking procedure, could be accounted for by percentage of total glutenin in flour.

The work of Autran et al. (1987) would suggest that in durum wheats low HMW:LMW glutenin subunit ratio results in superior pasta quality. Durum glutenin is composed of approximately 80% LMW subunits (Payne et al. 1984). Feillet and co-workers (1989) concluded that the contribution of LMW-GS to pasta firmness and viscoelasticity was due to its ability to aggregate upon heat treatment, such as during high temperature drying (>70°C) and cooking in boiling water. They also theorized that sulfur-rich glutenins found in durum by Kobrehel et al. (1988), contribute to LMW-GS, and possibly HMW-GS, aggregation through hydrophobic interactions and disulfide bonds.

Dough Rheology

Traditional empirical rheological methods that were originally developed for use in bread dough testing, such as the farinograph, mixograph and alveograph, have been adapted for measurement of durum wheat semolina gluten strength to provide practical information to processors (Irvine et al 1961, Quick and Donnelly 1980, Walle and Trentesaux 1980). The alveograph test has become widely accepted by commercial durum wheat processors and is used in international trade specifications (Dexter et al 1994). The alveograph subjects dough to biaxial extension, thus providing information on both dough strength and extensibility.

The mixograph is commonly used in the United States for assessment of durum wheat quality. Dough samples are prepared on a constant water absorption basis, and the resulting mixograms scored against a series of standard curves (Quick and Donnelly 1980). There is disagreement as to the ability of the mixograph method to predict pasta cooking quality (Dick and Quick 1983, Matsuo et al 1982).

Irvine et al (1961) developed a farinograph method using water absorption levels that approximated pasta commercial processing conditions (27% to 35%), measuring dough development times and tolerance to prolonged mixing. Long dough development times and a high tolerance to prolonged mixing were predictors of superior pasta cooking quality. Semolina protein content, farinograph bowl temperature, semolina particle size

and milling extraction rate affected farinograph results (Irvine et al 1961, Dexter and Matsuo 1978). Dexter and Matsuo (1980) have since recommended using only farinograph bandwidth because it is less sensitive to variations in protein content and correlated well with pasta cooking quality.

Dynamic rheological analysis provides an additional means of studying the physical properties of gluten and its various fractions. Several review articles have summarized the abundance of experimental evidence that wheat flour dough and gluten exhibit viscoelastic properties (Baird and Labropoulos 1982, Eliasson 1990, Faubion and Hoseney 1990). Baird and Labropoulos (1982) focused on those studies leading to well defined material functions and application of viscoelastic models to food doughs in general. They noted that food doughs exhibit both storage and loss moduli in small amplitude oscillatory testing that may be used to provide information about the nature of intermolecular interactions. In addition, they identified stress overshoot at the start up of flow, stress relaxation at the cessation of flow, viscosity that depends on shear rate, and normal stress differences as characteristic of food doughs. They also stressed that the chemistry of food systems was far more complex than for synthetic polymeric systems such as polymer melts, in which the viscoelastic properties are attributable to entanglement networks.

Moisture content of dough affects rheological measurements. Wheat milling technique can have profound effects on the degree of starch damage for hard wheats that, in turn, affects water absorption. Therefore, milling conditions must be controlled to minimize

starch damage. Generally, because of the milling techniques used, durum semolina has low levels of starch damage. As a result, variations in semolina water absorbing capacity are largely attributed to protein content (Quaglia 1988).

Under dynamic measurement conditions, increasing moisture content of dough results in a shift of the moduli to lower values (Dreese et al 1988, Edwards et al 1996, Navickis et al 1982). The ratio of the moduli (tan δ), however, is not affected by dough moisture content (Berland and Launay 1995, Dreese et al 1988). Campos and co-workers (1997) attempted to separate the effects of hydration and energy input by comparing developed and undeveloped hydrated doughs. They found that undeveloped dough still exhibited linear viscoelastic behaviour but with lower creep compliance and lower complex modulus than developed dough. Schluentz and co-workers (2000) demonstrated that energy input is necessary to facilitate interaction or bonding of wheat proteins leading to formation of a matrix structure.

Hargreaves and coworkers (1996) proposed that gluten viscoelasticity in common wheats is controlled by both the size of glutenin polymers and by the density of low energy bonds. Size of glutenin polymers is controlled primarily by HMW glutenin composition. They suggested that density of low energy bonds, composed of hydrogen bonds plus entanglements and hydrophobic interactions, is related to the dough LMW glutenin composition.

The actual macromolecular structure of the glutenin polymer and how that structure affects rheological properties is still not known for certain. Wrigley and co-workers (1998) have summarized gluten protein-protein interactions and have suggested possible relationships to dough rheology. Greenwood and Ewart (1975) proposed that entangled long linear molecules were formed during dough mixing by end-to-end linking of individual polypeptides via disulfide bonds, leading to the rubber-like elastic character of dough (Muller 1969). Bloksma (1975) proposed that the number of rheologically effective disulfide cross-links affects dough elastic deformation, and that viscous flow occurred during breaking and reformation of disulfide linkages via thiol-disulfide interchange reactions. Gluten and gluten fractions have been described as exhibiting characteristics typical of a transient network (Cornec et al. 1994, Hargreaves et al. 1996). These descriptions are similar to those of a physical gel, containing both entanglements and cross-links whose reversible junctions exhibit highly enhanced viscoelastic properties (Tanaka and Edwards 1992a).

Belton (1999) proposed a recent hypothesis for gluten elastic behaviour. He described a "train and loop" mechanism in which glutenin subunits, end-linked by disulfide bonds, form interchain associations via hydrogen bonding. Areas that are involved in bonds form the "trains" and unbonded regions form the "loops". Working or stretching the dough extends the loops and the trains are pulled apart allowing the proteins to slide along one another. Re-establishment of the train-loop equilibrium provides elastic recovery. The "train and loop" mechanism is primarily reliant on HMW glutenin content. Theories that assume gluten strength is primarily due to entanglements

involving the highest molecular weight fraction (for example, Tsiami et al 1997, Weegels et al 1996) describe behaviour similar to that of an uncross-linked polymer melt with entanglements (Gupta 2000).

There are relatively few studies that have determined linear viscoelastic properties of doughs or gluten through creep or stress relaxation testing. Early creep experiments indicated that gluten gels can be considered as an ideal elastic material at shorter times, viscous at longer times, with an intervening period of retarded elasticity (Funt Bar-David and Lerchenthal 1975, Hibberd and Parker 1979). Bohlin and Carlson (1981) found that gluten gels relaxed stress completely in about 10,000 seconds. Using stress relaxation techniques to study common wheat doughs, Rao et al (2000) found that extra-strong common wheat lines exhibited bimodal relaxation spectra, while moderately strong common wheat lines exhibited only a single prominent peak and a shoulder. Addition of cysteine to the extra-strong dough resulted in relaxation behaviour similar to that of the moderately strong line without any added cysteine. The second peak of the relaxation spectra was reduced to a shoulder, leading to the conclusion that high molecular weight glutenins were responsible for the second peak of the relaxation spectra and for the greater gluten strength of extra-strong dough. Rao and co-workers (2001), again using stress-relaxation techniques, found that stronger durum doughs took longer to relax to 50% and 75% of their initial stress, regardless of their initial relaxation modulus value, than weaker durum doughs. They also found strong correlation between relaxation times and extensigraph R_{max}/E, alveograph P/L and loaf volume.

Starch granule surface properties and interaction with gluten may influence dough rheological properties. Larsson and Eliasson (1997) studied the influence of starch granule surface properties on rheological behaviour of wheat flour doughs by diluting a flour with treated and untreated starch granules to a fixed protein content. The treatments used included heat treatment of starch and adsorption of protein on starch granule surfaces. Seguchi (1984) previously demonstrated that heat treatment of starch shifts the native granule surface from hydrophilic to hydrophobic. Larsson and Eliasson (1997) found that addition of heat-treated starch resulted in higher storage and loss moduli in dough, and led to longer relaxation times. The change in surface properties of heat-treated starch may have resulted in increased affinity of the now hydrophobic granules to hydrophobic gluten. An alternative explanation was that addition of water to the hydrophobic heat-treated starch in dough resulted in increased aggregation of starch granules. They stated that the presence of particle aggregates in a continuous matrix should lead to increased G' with an accompanying instability toward increasing strain, both of which were observed in the doughs containing heat-treated starch. Protein adsorption on starch granules increased dynamic moduli and led to longer relaxation times, with a slight increase in strain tolerance (the response of the moduli to increasing strain) relative to native starch, suggesting a stronger interaction of the granule surface with the gluten phase (Larsson and Eliasson 1997). The conclusion was, therefore, that interactions between starch granule surface and gluten can influence rheological behaviour of wheat flour dough.

Protein Enrichment/Reconstitution Studies

A simple, elegant means of investigating structure/function relationships of various protein components is by incorporating protein into a base flour or semolina and examining effects on dough mixing characteristics. Until the recent development of the 2g mixograph, studies involving isolated subunits were impracticable because of the relatively large amounts of protein required. Development of the micro-mixograph along with protein isolation and incorporation methodology opens up vast possibilities for studying the effects of constituents normally found in minute quantities. Bekes et al. (1994a) have developed a means of chemically incorporating added protein subunits into the glutenin polymer using controlled reduction/oxidation with a 2g mixograph. Reducing agents acted to disrupt intermolecular disulfide bonds, creating sulfhydryl groups. The glutenin subunit to be incorporated was added to the dough mix. Addition of oxidizing agents resulted in cross-linking of sulfhydryl groups into intermolecular disulfide bonds, incorporating the added fraction. Dithiothreitol (DTT) and potassium iodate were found to be the most effective combination of reducing and oxidizing agents in restoring original dough mixing characteristics. Using controlled reduction followed by re-oxidation, incorporation of glutenin subunit 1Bx20 from durum wheat led to increased mixing time, peak resistance and resistance to over mixing, whereas simple addition resulted in shorter mixing time, reduced peak resistance and more rapid breakdown (Bekes et al. 1994b). The results from incorporation were in agreement with statistical predictions as to the action of this subunit. One must bear in mind, however, that in using the reduction-oxidation method there is no control over where the added

subunit is incorporated or over the number or placement of re-established or created disulfide bonds. In fact, it appears from work done by Uthayakumaran and co-workers (2000b) that strength and extensibility characteristics are significantly altered and must be optimized for each cultivar being studied. Even once "optimized", the original dough characteristics were not completely recovered and extension curves were altered. Veraverbeke et al (1998) demonstrated that simple addition of HMW-GS strengthened dough, without going through the reduction-oxidation procedure during mixing. Addition of alkylated HMW-GS had no effect on dough mixing properties. Alkylation blocked any free sulfhydryl groups in the HMW-GS. The fact that alkylated and unalkylated HMW-GS behaved differently when added to dough suggests that unalkylated HMW-GS can be incorporated into endogenous glutenin polymer and that free sulfhydryl groups play an important role in glutenin polymerization during dough mixing.

One of the challenges of reconstitution methodologies is to isolate and purify protein without altering functionality. Skerritt et al. (1996) systematically looked at different methods of preparation of glutenin and gliadin fractions to determine the effects on functionality relative to control samples. A major conclusion of the study was that it was necessary to use dilute acetic acid rather than water for dialysis to maintain functionality of gluten protein fractions. It was suggested by Weegels et al. (1994) that acid prevents aggregation during dialysis. A range of extracting solvents could be used, excluding reducing agents, as long the samples were dialyzed against a weak acetic acid solution (pH~6) to maintain functionality in mixing studies. Treatment of glutenin

fractions with more concentrated acetic acid solution (0.1M) did result in reduced mixing strength. It would appear that it is important to maintain fraction pH close to that of dough which has been measured to be approximately 6 (Hoseney and Brown 1983) in order to get a true indication of functional properties of protein fractions in mixing studies.

Cross contamination of glutenin with gliadin, particularly ω-gliadin, has been a problem when attempting to isolate and purify glutenin. Fu and Kovacs (1999) developed a method using 0.3M NaI in 7.5% 1-propanol to effectively remove gliadin from gluten that may be used prior to glutenin isolation to ensure purity of the isolate. Methods to further fractionate glutenin into HMW-GS and LMW-GS have been developed based mainly on their solubility in 1-propanol with inclusion of a reducing agent, followed by selective precipitation by increasing the concentration of 1-propanol (Marchylo et al 1989, Verbruggen et al 1998).

Starch

Although starch constitutes the largest volume fraction of solids in dough, comprising approximately 75% (Colonna et al. 1990), limited work has been published describing the contribution of starch to dough rheological properties. Rasper and deMan (1980) looked at the effect of granule size on dough tensile stress-strain curves. They prepared wheat flour doughs supplemented with starches from a variety of botanical sources and with glass powder of similar particle size distribution. While they were unable to

conclusively establish a fundamental relationship, they were able to demonstrate that particle size did have an effect. They also found differences between dough rheological response to added glass powder and starch of a comparable particle size, dependent on botanical source of the starch. They concluded that factors such as water-binding capacity of the starch might play more of a role in dough rheological properties than specific surface area of the granules. In contrast, Lelievre et al (1987) using starch segregated into three particle size ranges, did not see any clear effect of starch particle size on water absorption. They suggested that the area over which gluten spreads, or specific surface area of granules, affected properties of bread made from gluten and starch doughs. Hayman and co-workers (1998) found that increasing the proportion of large starch granules had a sufficiently negative effect on gas cell wall stability of fermented doughs to result in coalescence of gas cells and open crumb grain in the baked product.

In a study on the effect of starch granule size distribution on extensigraph measurements Sebecic and Sebecic (1995a) stated that the presence of a greater proportion of granules ranging from 6.5 to 19.5 μm in diameter significantly increased dough extensibility and decreased resistance to extension. Very large granules (>27.5 μm) increased resistance to extension. Sebecic and Sebecic (1999) claimed that high content of very small starch granules (<6.5 μm) or large granules (>16 μm) caused weakening of dough mixed in a farinograph. In addition, they stated that granules representing the largest proportion of native granules, in the range of 6.5 to 16 μm, had a positive effect on dough

development time and stability. All of their studies were correlative in nature, having previously determined starch granule size distribution in a series of flour samples using a polarizing microscope (Sebecic and Sebecic 1995b). They performed statistical analyses relating various rheological parameters to ranges of granule distribution. Their results, however, are suspect because they did not take into account the effect of protein content, which ranged from 8.7 to 14.0%, on dough mixing and extensibility properties.

Seguchi (1993) demonstrated that chlorination led to starch granule surfaces becoming hydrophobic, and altered the textural characteristics of pancakes relative to native starch. Heat treatment of starch granules at low moisture content has also been shown to generate a hydrophobic surface and decreased susceptibility to α-amylase and acid hydrolysis (Hoover and Vasanthan 1994, Seguchi and Yamada 1988).

Protein-Starch Interactions

It is not realistic to study protein or starch in isolation and expect to be able to explain the complete dough strength story. A materials science approach should assist in the exploration of the overall concept of dough rheological properties, taking into consideration the various major components. The field of materials science has been borrowed and adapted from classical engineering and applied to the study of food materials. The goal of this approach is to measure the size and distribution of components in a heterogeneous mixture, determine how these components interact, and from this information develop processes that will produce optimized structures and

properties of the desired product (Stanley 1994). Dough may be viewed as a heterogeneous composite material, analogous to a filled polymer composite, composed of a protein matrix with embedded starch granules. In addition there are several minor components in dough including lipids, non-starch polysaccharides and minerals that will not be discussed here. The properties of the composite should depend on material properties of the protein matrix and starch filler, the total volume occupied by starch granules, size distribution of starch granules and any interaction between them, the presence of any voids in the structure, and degree of adhesion and interaction between the starch granules and protein matrix. Fillers may be either reinforcing or nonreinforcing, depending on whether or not there is adhesion between the matrix and filler. Two extremes in behaviour of composite materials consisting of a dispersed particle filler and a gel matrix were identified by van Vliet (1988), depending on the nature of the filler and the matrix. First, where there is no interaction between the matrix and the filler, moduli decrease with increasing volume fraction under small deformation test conditions. Where there is strong interaction between filler and matrix, there will be an increase in moduli with increasing volume fraction of the filler material if the filler particles are stiffer than the matrix.

There is some disagreement as to whether starch granules act as reinforcement to the protein matrix. Amemiya and Menjivar (1992) state that because of its very high concentration starch may form or be part of the continuous phase. There is not, however, a reliable molecular theory for filler reinforcement in general, describing the

origin of reinforcement, even in the study of rubberlike networks (Erman and Mark 1997).

A materials science approach to explaining the relative contributions of the various components in determining dough strength, mixing or processing characteristics has been very limited. Amemiya and Menjivar (1992) using this approach, suggested that starch-starch and starch-protein, as well as protein-protein interactions may be important sources of elasticity in doughs, and that the effects would be most observable at small deformations because of their short-range nature. The authors were unable, however, to satisfactorily resolve the relative contribution of these interactions using dynamic oscillatory measurements. Comparing bread and biscuit flour doughs, the authors found that the biscuit flour dough had a higher complex modulus (G*) than the bread flour dough while maintaining similar tan δ. This was attributed to the fact that biscuit flour had higher concentration of starch (56% versus 51%) and lower moisture content (36% versus 39%). These flours, however, also had a 3% difference in protein content which should not be ignored or under-rated, as protein content has been shown to affect dynamic moduli (Edwards et al 1993). In the study by Amemiya and Menjivar (1992) the three variables being compared (protein, starch and moisture content) were not consistent among samples used. Using large deformation measurements at strains varying from 1% to 3000%, they identified starch-starch and starch-protein interactions as being dominant at strains less than 300%. At strains greater than 300% protein-protein interactions resulting from the continuous protein phase network dominated,

provided that there was sufficient protein content and network development. In the absence of a continuous protein network, in this case from biscuit flour dough with 6% protein, viscoplastic behaviour was observed under large deformation, whereas bread flour dough at 9% protein displayed elastomeric behaviour.

Petrofsky and Hoseney (1995), using commercial gluten and starch from soft wheat, hard wheat and nonwheat starch, prepared doughs and compared their rheological response. Doughs made from soft wheat and nonwheat starch sources had higher moduli than hard wheat starch or commercial starch. They suggested that there was greater interaction between the gluten protein and both the soft wheat and nonwheat starch, leading to higher moduli.

Walberer and McHugh (2001), working with polydimethylsiloxane (PDMS) filled with glass beads, found that molecular weight of the polymer matrix played an important role in determining the dependence of tan δ on filler volume fraction, particularly at frequencies below 10 Hz. As polymer molecular weight increased, tan δ became nearly independent of the filler volume fraction over the frequency range studied (0.1 to 100 Hz). The PDMS polymers that they used ranged in molecular weight from 100,000 to more than 2,500,000 and the glass beads had an average diameter of 7 μm, with 90% finer than 15 μm. The tests were conducted using blends ranging from unfilled PDMS up to 60% filled. The effect of increased filler volume fraction was to increase the elasticity of the material for lower molecular weight polymer as indicated by tan δ. The molecular weight of the PDMS was approaching that of glutenin, estimated as being up

to 11 million (Wahlund et al 1996), and the glass beads would be similar in diameter to those of small starch granules, so there may be similarities to be drawn to dough.

In the classical fields of engineering a number of equations and theories have been developed to explain or describe interactions between components of heterogeneous mixtures, for example particle filled polymer resins. Ahmed and Jones (1990) reviewed many of the relevant theories and equations. Although the authors identified several problems, some of these theories may be applicable to wheat flour doughs, considering gluten networks to be analogous to the polymer matrices and starch granules to the particulate fillers. The simplest equations for describing the modulus (E) of a two phase composite material were given by the parallel model:

$$E_c = E_p V_p + E_m V_m \qquad (1)$$

And by the series model:

$$E_c = (E_p E_m)/(E_p V_m + E_m V_p) \qquad (2)$$

Where V represents the relative volume fractions and the subscripts denote that c is the composite, p is the particulate filler, and m is the matrix. Equation (1) assumes that there is uniform strain in both phases, and equation (2) assumes uniform stress across both phases inferring strong adhesion between phases and no aggregation of the filler particles. There is also the assumption that the modulus of the composite is dependent

solely on volume fraction and that particle size variation has no effect. Ahmed and Jones (1990) cited several studies that found an increase in modulus with decreasing particle size. Both of these approaches are probably too simplistic for a wheat flour dough as starch granules vary in size, but may be worth examining as a starting point. Variations of these equations developed by Ishai and Cohen (1967)

$$E_c = E_m (1 + (V_p / (m/(m-1) - V_p^{1/3})) \quad (3)$$

and by Paul (1960)

$$E_c = E_m((1+(m-1)V_p^{2/3}) / (1 + (m-1)(V_p^{2/3} - V_p))) \quad (4)$$

where $m = E_p/E_m$

were found by Ahmed and Jones (1990) to better describe actual data of samples containing volume fractions of glass beads varying in size that were embedded in epoxy resin. They also suggested that equations 3 and 4 may have value in describing composites with agglomerated particles or where there may be formation of a filler-matrix interphase. Both of these equations assume that the constituents are in a state of macroscopically homogeneous stress and that there is adhesion at the particle-matrix interface. These equations may be of some value in describing wheat flour doughs, but they generally apply to composites where the proportion of filler is relatively small, which may pose problems in their application to dough.

There has been some work in the field of polymer engineering on measuring interfacial bond strength at the fiber-matrix interface of composite materials. The techniques may have potential application in studying starch granule-protein interface bonding or adhesion. According to Edie and co-workers (1991), in composite materials consisting of essentially elastic fibers embedded in a viscoelastic polymeric matrix, deformation energy is mainly dissipated in the matrix and at the matrix-fiber interface. If there is poor interfacial bonding, more energy is dissipated than if there is strong interfacial bonding.

Edie and co-workers (1991) were able to separate the contributions of interfacial bonding in epoxy-fiber composites from those of the matrix and fibers using dynamic mechanical analysis. Three different types of carbon fibers were used, but all were essentially elastic. They were able to demonstrate that in comparison to neat epoxy, the loss modulus (G") increased three to six times with the inclusion of carbon fibers at a constant volume fraction of 60%. The fibers used were highly elastic and did not generate viscous losses. Therefore, the increase in G" was attributable to the properties of the fiber-matrix interface in the composites.

The same researchers (Edie et al 1991) prepared a series of samples using a single fiber type with varying volume fractions (0% to 60%) to provide a range in fiber-matrix interfacial area in an epoxy resin-fiber composite. They determined that G', G" and tan

δ increased with increasing volume fraction of carbon fiber, and that G" and tan δ appeared to correlate well with total interfacial area.

Summary

There is still incomplete understanding of the mechanisms involved in dough viscoelastic behaviour. Most of the attempts to relate protein structure to dough functionality, and theories to explain the mechanisms involved, have been developed based on common wheat performance and processing requirements, which are quite different from those of durum wheat. With the development of reliable protein fractionation procedures that cleanly separate the various fractions, and micro dough processing procedures, studies may now be undertaken to evaluate the role of each of these fractions in dough viscoelastic properties. Fundamental rheological testing provides a powerful tool for probing the underlying molecular processes that dictate dough responses to deformation during processing. In addition to the information gained through testing of protein fractions, fundamental rheological testing affords the opportunity to determine the role of the starch granule-gluten protein interface in dough viscoelastic properties. Application of the techniques and theories developed primarily in polymer engineering provide a sound starting point for development of hypotheses explaining the mechanisms involved in dough elastic behaviour, and its ability to flow in response to applied stress and to recover following cessation of stress.

CHAPTER 3

Relationship of Creep-Recovery and Dynamic Oscillatory Measurements to Durum Wheat Physical Dough Properties[1]

Abstract

Durum wheat gluten strength is important in determining extrusion properties and pasta cooking quality. Durum wheats varying in strength were tested using an Alveograph and a 2g micro-mixograph, both widely accepted techniques for determination of physical dough properties. Doughs from the 2g micro-mixograph were characterized by dynamic oscillatory and large deformation creep tests using a controlled stress rheometer. Mechanical properties obtained from both testing regimes were strongly correlated with many of the parameters provided by the Alveograph and micro-mixograph. Maximum strain attained after 5 minutes creep ranged from less than 5% for the strongest least extensible cultivar to over 25% for the weakest cultivar, with a coefficient of variation (CV) among replicates of less than 10%. Storage modulus (G'), at 2 Hz, ranged from about 7000 Pa for the weakest cultivar to over 16000 Pa for the strongest least extensible cultivars, with a CV of less than 6%. Tan δ (G"/G') values were near 0.4 for the strongest versus more than 0.5 for the weakest cultivars, indicating the larger contribution of the elastic component in the strong cultivars. The rheometer allows discrimination of durum wheat cultivars of varying gluten strength while requiring less sample than traditional physical dough testing techniques.

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Introduction

Extrusion properties of durum wheat semolina doughs and pasta cooking quality are largely determined by gluten strength of the raw material. Several chemical and instrumental methods have been developed to assess gluten strength with attempts to relate data from these methods to processing characteristics and end-product quality (Cole 1991). Traditional empirical rheological methods used in bread dough testing, such as farinograph, mixograph and alveograph, have been adapted for monitoring of durum wheat semolina gluten strength to provide practical information to processors (Irvine et al. 1961, Quick and Donnelly 1980, Walle and Trentesaux 1980). Alveograph, which subjects doughs to biaxial extension, has become the most widely accepted instrument by commercial durum wheat processors and is used in international trade specifications because it provides information on both dough strength and extensibility (Dexter et al. 1994). The alveograph requires relatively large amounts of semolina (250 g), which can be a hindrance where sample size may be a limiting factor, such as in screening of plant breeders lines or in reconstitution experiments.

Development of a 2g direct drive mixograph, or micro-mixograph, interfaced with a personal computer for data collection and interpretation has provided a means of establishing traditional empirical mixing characteristics where sample size is limited (Rath et al. 1990). Khatkar and co-workers (1996) used the micro-mixograph and a small-scale baking procedure to evaluate the baking potential of flours from wheats of

diverse genetic origin. They concluded that the micro-mixograph may be a valuable alternative to baking trials in assessing flour baking potential. A limitation of the micro-mixograph is its inability to provide information on dough extensibility.

Kieffer et al. (1998) developed a micro-scale extension test for dough and gluten, similar to an extensigraph test using doughs prepared with 2% salt and mixed to a constant consistency in a 10g farinograph. They found that extension results, in combination with flour protein or wet gluten content, could reliably predict loaf volumes of micro-scale baked bread.

Several techniques have been employed for investigating the fundamental mechanical properties of wheat flour doughs, including stress relaxation (Fu et al. 1997, Launay and Buré 1974), creep and creep recovery (Campos et al. 1997, Hibberd and Parker 1979) and dynamic oscillatory measurements (Abdelrahman and Spies 1986, Amemiya and Menjivar 1992, Faubion and Hoseney 1990). Dynamic oscillatory measurements have been most commonly used to assess fundamental mechanical characteristics of wheat flour dough, however, there is no consensus as to their practical advantage over established empirical methods. Using dynamic oscillatory measurements, Janssen and co-workers (1996) concluded that there was no direct relationship for either dynamic moduli or tan δ to loaf volume, but that the information obtained was complementary to biaxial extension and other rheological measurements. Kokelaar et al. (1996) suggested that biaxial extension tests were more relevant to breadmaking quality, and that small

strain dynamic measurements were not related to biaxial extension measurements. Huang and Kokini (1994) measured biaxial extensional viscosities and related these measurements to loaf volume development during proofing. Subsequently, Huang and Kokini (1999) linked biaxial extensional measurements to loaf volume expansion during proofing. Campos and co-workers (1997) used dynamic oscillatory and creep measurements on undeveloped doughs and doughs prepared by mixing in a farinograph bowl in an attempt to decouple the effects of hydration and energy input. They reported that undeveloped doughs exhibited lower creep compliance and lower complex modulus (G*) values than developed doughs. They also found that hard wheat dough was more viscous and much more resistant to deformation than soft wheat dough, consistent with their farinograph data which showed hard wheat dough to be stronger with much longer stability and lower mixing tolerance index than soft wheat dough.

Our objective was to establish a small-scale method that would provide information on durum semolina dough strength and extensibility and relate to traditional empirical methods. Dynamic rheometers require relatively small samples and are capable of providing information under conditions of small and large deformations, but to date the limiting factor determining sample size has been the mixers used to prepare the dough. The advent of the micro-mixograph has provided a means of working with small (2g) dough samples. Factors considered in the study included the effect of semolina protein content, wheat variety and environment.

Material and Methods

Wheat

Composites of commercially grown durum wheat collected from a variety of locations in western Canada were prepared at four protein levels for each of three years (n=12). All samples graded No 2 Canada western amber durum (CWAD) or better. Varietal distribution within the composites was assessed by electrophoresis (Tkachuk and Mellish 1980) to confirm relatively consistent distribution of varieties among samples. All samples contained on average 65% of the cultivar Kyle, which is the proportion of Kyle found in the CWAD crop during these years (Canadian Grain Commission, unpublished data).

Preparation of composites for each of 3 crop years with differing annual growing conditions allowed us to consider the effect of environment as well as protein content. Variations in protein within each crop year may arise from micro-climates that had their own unique effects. Areas producing either extremes of high or low protein wheat tended to be very localized, and may have had different climatic and soil fertility conditions from the rest of the growing area.

Wascana, Kyle, Durex, and AC Melita were grown at the Agriculture and Agri-Food Canada (AAFC) Semiarid Prairie Agricultural Center in Swift Current, Saskatchewan in

1996 and at AAFC field plots in Regina, Saskatchewan in 1997. Additional plots of Stewart 63 were grown in 1997 at AAFC Swift Current.

The varieties Kyle and Wascana were representative of moderate strength and relatively extensible varieties grown in western Canada for the past thirty years. AC Melita is a new CWAD variety with stronger gluten, registered in response to market demand for greater gluten strength in durum wheat. Durex is a so-called "desert durum" variety with very strong gluten, developed for production in south-western United States. The additional strength of AC Melita and Durex is evident from higher alveograph P/L and W values, and longer farinograph development times and stabilities, than moderate strength CWAD varieties.

Stewart 63 was added to extend the range in dough strength. Stewart 63 is a γ-gliadin 42 type durum wheat, that is no longer registered to be grown in western Canada because of its inferior pasta making characteristics and very weak dough mixing properties. Wascana, also a γ-gliadin 42 type, represents the extreme high end of dough strength observed in γ-gliadin 42 types. All other varieties were γ-gliadin 45 types. The presence of γ-gliadin band 42 has been associated with poor pasta quality, while band 45 has been associated with superior quality (Damidaux et al. 1978).

Italian varieties Creso, Grazia, and Simeto were supplied by Instituto Nazionale della Nutrizione, Rome, Italy from the 1996 Italian harvest.

Milling

All wheats were cleaned and tempered overnight to 16.5% moisture content. Commercial composites were milled using a four-stand Allis-Chalmers laboratory mill (Allis-Chalmers, Milwaukee, WI) in conjunction with a laboratory purifier (Black 1966) using the procedure of Dexter et al. (1990). The milling area was controlled for temperature (21°C) and relative humidity (60%). Semolina yield ranged from 65.0% to 70.8% of clean wheat on a constant moisture basis, with a typical particle size distribution of 6% held on 420μ sieve, 65% on 250μ, 19% on 177μ, 6% on 149μ and 4% through 149μ.

Semolina Tests

Analytical results were expressed on a 14% mb for semolina. Moisture content was measured by single stage air oven AACC (2000) Method 44-15A. Protein content was determined by combustion nitrogen analysis (CNA) using a LECO Model FP-428 CNA analyzer (Leco Corp. St. Joseph, MI) calibrated against ethylenediaminetetraacetic acid (EDTA). Analysis for starch damage was by the Megazyme AACC (2000) Method 76-31.

Physical Dough Tests

Alveograph curves were obtained using the constant pressure Model MA82 (Chopin SA, Villeneuve-la-Garenne, France) following ICC (1980) Standard No. 121. Values for P (maximum peak height), L (length of the curve) and W (work of deformation until rupture) were automatically calculated by the instrument.

Mixograph data were obtained using a 2g direct drive mixograph (National Manufacturing Division, TMCO, Lincoln, NE) at fixed water absorption of 50% on 14% mb, resulting in a final dough moisture content of 42.7%. Samples and water were weighed to 0.001g. The temperature of the mixing bowl was maintained at 25°C. Tests were performed in triplicate. Micro-mixograph parameters were determined using "Mixsmart" computer software provided with the instrument using mid-line analysis.

Farinograph (C.W. Brabender Instruments, South Hackensack, NJ) mixing characteristics were obtained using AACC (2000) Method 54-21.

Rheological Testing

A Rheometric dynamic stress rheometer model SR500 (Rheometric Scientific, Piscataway, NJ) was used in both oscillatory tests (strain controlled) and creep (constant stress) in shear mode. The rheometer was equipped with 25mm diameter serrated upper and lower parallel plates that were maintained at 25°C.

Initially samples were prepared by mixing at 50% absorption on 14% mb in the 2g mixograph to establish peak mixing time. Preliminary rheological testing of mixograph doughs using samples mixed to peak gave unsatisfactory precision in the resulting rheological data (data not shown), probably because peak mixing times varied by up to half a minute among replicates. Subsequently, when doughs were mixed to 1 minute past peak precision improved. Mixing to 1 minute past peak was therefore adopted for preparation of dough for rheological testing.

A sample of dough was removed from the bowl, a 2g portion was rounded by hand, and placed between the plates of the rheometer. The sample was gently flattened to fit the plate geometry using a teflon coated spatula and the upper plate lowered to a fixed gap of 2.75mm. Exposed edges of dough were liberally coated with mineral oil (Sigma Chemical Co., St. Louis, MO) to prevent drying.

Strain sweeps conducted at 1 Hz indicated linear response at strains between approximately 0.25 and 0.6%, in agreement with previously published data (Amemiya and Menjivar 1992, Dus and Kokini 1990, Lindahl and Eliasson 1992). Subsequent oscillatory measurements were taken at 0.5% strain over a frequency range of 0.5 to 10 Hz.

Kyle and AC Melita semolina samples were used to determine rest times required prior to frequency sweeps, as they represent the typical range of dough strength found in commercially grown durum wheat in western Canada. Oscillatory measurements were

taken immediately after loading on the rheometer and every 5 minutes thereafter to a maximum of 30 minutes on 3 separately prepared doughs of each variety. Data obtained were storage modulus (G'), shear loss modulus (G"), complex modulus (G*), and tan δ (G"/G'), although only G' and tan δ are discussed. Statistical comparisons were made at 2 Hz, which is approximately the midpoint in frequency sweeps. As will be shown later, results indicated that a rest time of 15 minutes after loading in the rheometer and coating with oil was sufficient to achieve reproducible results, and was adopted as the standard rest time for subsequent analyses.

Creep-recovery tests were conducted immediately after oscillatory measurements on the same dough sample. Dynamic oscillatory testing at low strain is non-destructive and would not have caused changes to the sample structure. A stress of 100 Pa, which exceeded the region of linear viscoelasticity, was applied for 300 s, sufficient for the sample to reach steady state flow as determined by the instrument software (which allows for 10% slope variation). Creep tests performed at large deformation were essentially micro-scale shear extension tests. Results reported are the average of three replicates, where each replicate represents separately mixed dough. Tests, including rest time, frequency sweeps and creep to maximum % strain, were completed in under 30 minutes.

Water Absorption

Effect of water absorption on rheological data was determined using a set of pure varieties from a single location. They were prepared in the micro-mixograph at two additional water absorptions, 48% and 52%, for oscillatory and creep testing as described above. Semolina and water amounts were controlled so as to maintain constant dough weight of 3 g in the micro-mixograph bowl.

Experimental Design and Statistical Analysis

Analytical data were the average of duplicate tests. Micro-mixograph testing was conducted in randomized complete block design, in triplicate, and also expressed as averages. The commercial semolina samples and pure cultivars were tested as separate series. Oscillatory and creep-recovery testing were conducted in triplicate and the averages reported. Coefficients of variation (C.V.) were calculated for oscillatory and creep data. Alveograph data were reported as the average of five tests on a single batch of dough, while farinograph data were from single tests. All statistical analyses were performed using SAS (Cary NC) software as described in the appropriate SAS manual.

Results and Discussion

Development of Rheological Testing Protocol

Dough Resting Time

There is a great deal of variation in the literature concerning the amount of time required for dough relaxation after sample mixing prior to oscillatory or steady shear testing, ranging from 1 minute (Lindahl and Eliasson 1992) to 1 hour (Baltsavias et al. 1997). In some studies dough was rested after mixing and prior to loading, and then rested again after loading; for example Amemiya and Menjivar (1992) rested their samples for 2 hours prior to loading and then for 5 minutes after loading. Other workers rested samples after loading, and prior to testing. With so much diversity of sample handling in the literature, we decided to determine the effect of resting time after loading on the results of dynamic oscillatory tests, without having previously rested the dough in the mixer.

Ideally, rest times would be derived from the longest relaxation time obtained during stress relaxation testing. However, not having equipment capable of performing stress relaxation tests we determined the amount of rest time required beyond which no longer significant changes in G' and tan δ occur (Fig. 3.1).

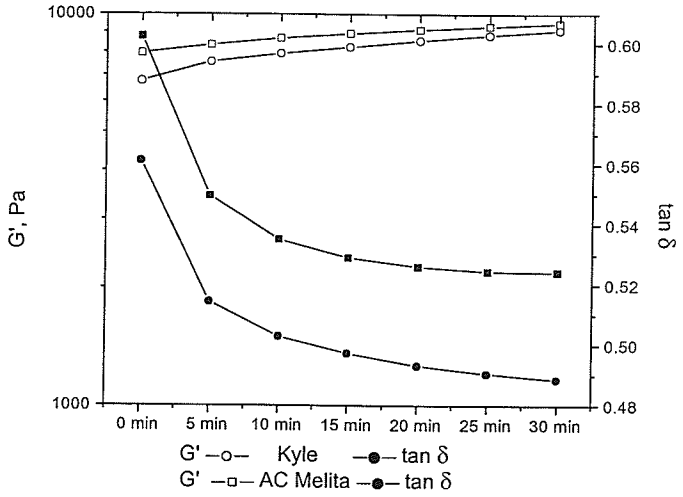

Figure 3.1 Relationship between dough rest time after sample loading between dynamic rheometer parallel plates and dynamic oscillatory parameters of storage modulus (G') and tan δ (G"/G') for the durum wheat varieties Kyle and AC Melita.

Tan δ decreased and G' increased with resting time for both Kyle (moderate strength) and AC Melita (very strong). The two varieties were clearly differentiated throughout the complete range of rest times examined, and the pattern of change with rest time was similar for both. There was no statistically significant ($p>0.05$) change in either G' or tan δ beyond 10 minutes resting when compared at a fixed frequency of 2 Hz, although G' showed a continual increasing trend up to 30 min. Drying of the exposed surface of the dough would account for an increase in G', but we observed no evidence of drying up to 30 min rest time at ambient humidity. Lindahl and Eliasson (1992) also noted an increase in G' over time for durum wheat doughs, however, they reached plateau region at rest times beyond 20 minutes. Differences in rest time required to reach levels where there were no longer any significant changes may be due to differences in moisture

content. Lindahl and Eliasson (1992) were working with dough at 37% moisture content, whereas our dough was at 42.7% moisture. In addition, Lindahl and Eliasson (1992) used a farinograph for sample preparation, developing dough through kneading which is quite different from the elongation and rupture action of the mixograph. To satisfy a minimum requirement of 10 minutes, we chose 15 minutes as our rest time to ensure we were within the region where there would no longer be any significant changes in G' and tan δ.

Dough Moisture Content

Moisture content of dough is known to have an effect on dynamic mechanical results (Berland and Launay 1995, Edwards et al. 1996, Navickis et al. 1982). The ICC alveograph procedure (ICC 1980) recommends a fixed absorption level of 50%. Using a fixed absorption level is often a concern when working with flour samples because milling technique can have a profound effect on degree of starch damage, which in turn affects water absorption. As starch damage increases, dough becomes stiffer resulting in reduced alveograph extensibility, increased peak height and increased area under the curve (Dexter et al. 1994). Milling procedures used to produce semolina generally do not induce high levels of starch damage. Analyses for starch damage indicated that levels in our semolina samples were maintained at 4.7 Megazyme units or less. Variations in semolina water absorbing capacity are largely attributed to protein content (Quaglia 1988).

Samples of Kyle, AC Melita, Durex and Wascana were prepared at two additional absorption levels, 48% and 52%, to assess whether relative values for the different cultivars obtained by oscillatory and creep tests were affected by absorption. Farinograph absorptions of these four samples were within a relatively narrow range, from 56.8% to 60.4%. Kyle and Wascana were representative of the moderate strength, relatively extensible model for durum wheat grown in western Canada over the past several decades. In contrast, AC Melita and Durex, a variety from southwestern United States, are typical of stronger durum that has become more popular in recent years.

Variations in moduli due to mixing and loading of samples resulted in coefficients of variation of less than 5.5% between replicates. Under oscillatory test conditions there was a shift to lower moduli (G' and G") with increasing moisture content. This phenomenon has been well documented previously (Dreese et al. 1988, Edwards et al. 1996, Navickis et al. 1982). G' values ranked the samples in consistent order at each moisture content (Fig. 3.2). Differences in G' values for a given variety due to absorption were statistically significant ($p<0.05$). Tan δ values were not significantly ($p>0.05$) affected by moisture content (Fig. 3.3), in agreement with reports by Berland and Launay (1995) and Dreese et al. (1988). Although tan δ values were not significantly different at different absorptions, we observed significant ($p<0.05$) differences between varieties.

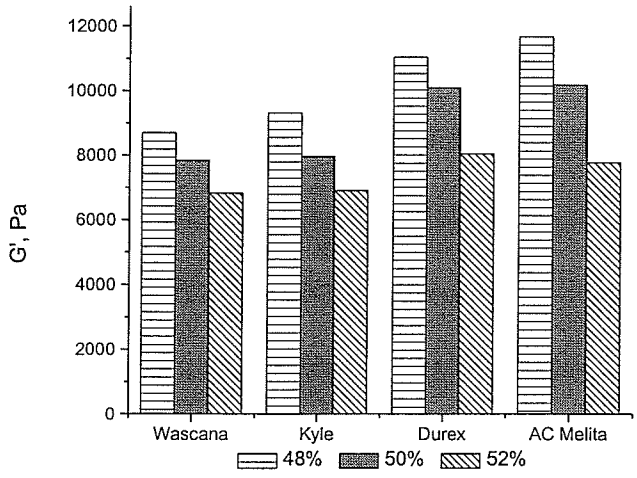

Figure 3.2. Effect of semolina water absorption on storage modulus (G') at 2 Hz for four durum wheat varieties.

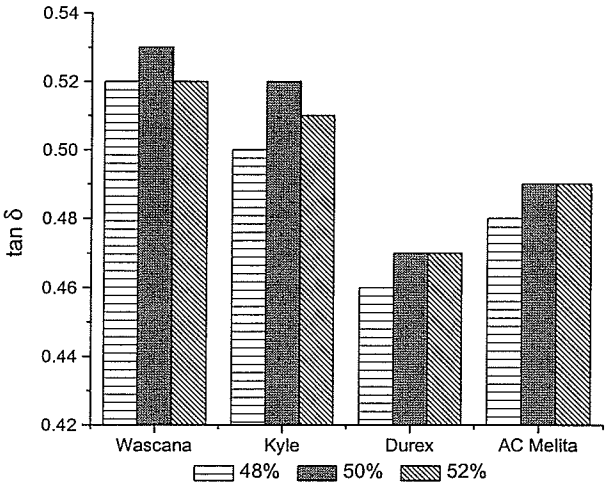

Figure 3.3. Effect of semolina water absorption on tan δ (G"/G') at 2 Hz for four durum wheat varieties.

Creep data at 48% and 52% absorption bracketed those of 50% for all four samples, ranking them in the same order at all absorption levels (Fig. 3.4). Increasing water absorption increased maximum strain attained, which one would expect, as adding

water would facilitate flow. Coefficients of variation were 9.7% or less among replicates. Differences in creep parameters were significant ($p<0.05$) at different absorption levels and among varieties at a given absorption level. Wascana was consistently most extensible, and Durex and AC Melita, which were indistinguishable from each other, were least extensible at all absorption levels.

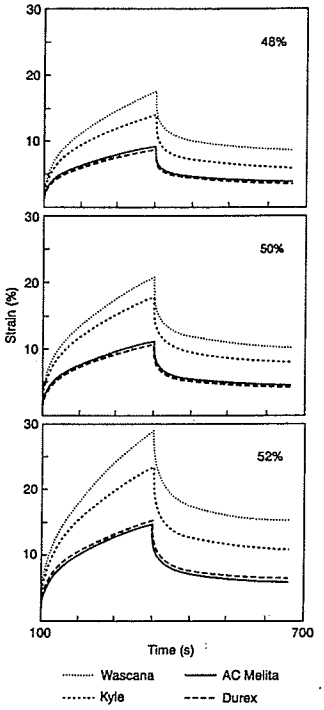

Figure 3.4. Creep-recovery, in shear, of four durum wheat varieties at three different semolina water absorption levels. Applied stress 100 Pa.

Confirmation that differences in G' and creep among varieties could not be explained based on water absorption made it reasonable to test samples at a fixed absorption. From a practical standpoint, we found that absorption levels higher than 50% resulted in doughs that were sticky and difficult to transfer to the rheometer, particularly for weaker cultivars. Since 50% is the recommended water absorption level for alveograph testing,

and the dough was relatively easy to handle, all further testing was conducted at a fixed absorption of 50%.

Creep Times

We used several creep times before deciding upon 300 seconds. Under the default criteria of our rheometer, which allowed for 10% slope variability and required a minimum number of 25 data points to fit a straight line, steady state flow was attained at 300 seconds. Using the cultivars Kyle, AC Melita, Durex and Wascana, we allowed doughs to creep for 300, 600, 900 and 10,000 seconds. In all cases the strength of the samples ranked in the same order (data not shown), and up to 900 seconds the curves indicated steady state flow (Fig. 3.5). At 10,000 seconds, however, the curves were no longer indicative of steady state. This may have been more a function of drying of the sample than a characteristic of the dough, as we observed formation of a "skin" at the exposed surface of the dough even though well coated with oil.

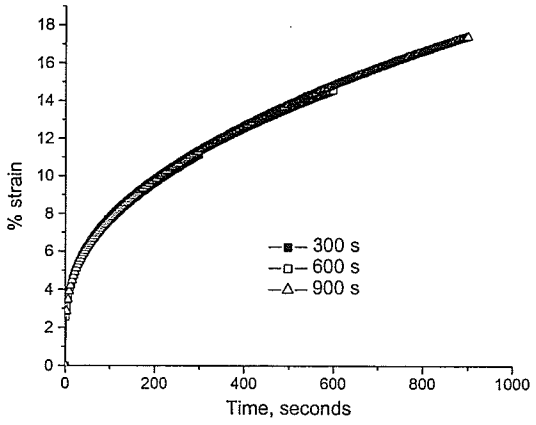

Figure 3.5. Creep tests conducted for 300, 600 and 900 s on separately prepared doughs in order to establish creep time required to attain steady state.

Three hundred seconds was chosen as standard creep time for succeeding experiments because not only was steady state attained, but the shorter time reduced the possibility of variability resulting from sample drying. Our stated objective was to develop relatively rapid small scale tests that related to traditional rheological tests used for screening of durum wheat. Consistent with that objective, we found that maximum % strain reached after 300 seconds was sufficient for discrimination of samples varying in strength.

Effect of Protein Content

Several studies have found that dynamic moduli of doughs are affected by flour protein content (Amemiya and Menjivar 1992, Lindahl and Eliasson 1992, Navickis et al. 1982), with higher protein content resulting in higher moduli. Hibberd (1970), using starch and gluten doughs, saw an increase in tan δ with increasing proportions of gluten. In contrast, Abdelrahman and Spies (1986) found a decline in tan δ with addition of gluten to flour. Generally, studies that compared doughs of variable protein content used wheats of diverse genetic origin which varied also in gluten strength, making it difficult to isolate effects solely due to protein content. Alternatively, studies that varied gluten to starch ratios did result in the ability to artificially isolate the effect of protein content, but at a fixed gliadin to glutenin ratio. Under field production, as wheat protein content increases the relative proportion of gliadin has been observed to increase (Dexter et al. 1989, Wieser and Seilmeier 1998). Gliadin is known to contribute to the

viscous nature of wheat doughs and to its extensibility (Fido et al. 1997, Orth and Bushuk 1972, Scanlon et al. 1990). To establish the effect of durum wheat protein content in its native form on dynamic moduli and large deformation creep we used composites of commercially grown Canadian durum wheat prepared over 3 years at 4 protein levels (high, medium high, medium low, low).

Farinograph absorptions increased with increasing protein content (Table 3.1), a well documented phenomenon (Tipples et al. 1978). There were no significant ($p>0.05$) differences in dough development time or stability resulting from differences in protein content. Micro-mixograph curves were affected by protein content. Lower protein samples generally had somewhat longer mixing times, lower peak dough resistance, narrower bandwidth at peak, more rapid breakdown, and lower work input requirements (Table 3.1), in agreement with work done by Khatkar et al. (1996). Alveograph data indicated reduced extensibility at low protein content, with the higher protein sample series having longer L values and lower P/L ratios (Table 3.1). High protein also resulted in increased strength indicated by higher W values. Dexter et al. (1994) demonstrated that increasing protein content in durum wheat resulted in increased alveograph extensibility (L), but had little effect on resistance to deformation (P) leading to lower P/L values and larger W values.

We were unable to detect a significant effect ($p > 0.05$) of protein content on G' by least significant difference (LSD). Environment had a very strong effect on G' ($p<0.001$).

Samples collected during the 1995 harvest had the highest G' values and 1997 the lowest.

Table 3.1. Rheological characteristics of protein composites, shown as the average for 3 years[a]

	High	Intermed High	Intermed Low	Low	F value Protein	F value Environ	F value P*E[b]
Protein, %	14.7	12.6	10.8	8.9			
Farinograph[c]							
Abs.,%	61.4a	59.5ab	57.8bc	56.2c	15.2*	2.0	
DDT, min	3.5a	3.4a	3.2a	3.2a	1.0	9.7*	
Stability,min	4.1ab	3.7b	4.4ab	4.7a	2.5	4.8	
2g Mixograph[d]							
MT, min	2.7b	2.5c	2.8b	3.0a	16.58**	1.04	4.40*
PDR, units	69.1a	57.2b	48.8c	42.8d	542.21**	71.18**	35.03**
MS, units	17.5a	14.4b	13.7bc	13.6c	59.20**	26.25**	3.82*
WI, %torque·min	131a	99b	99b	98b	38.28**	9.44**	3.64*
Alveograph[e]							
L, mm	92a	81ab	65bc	55c	4.91*	0.95	
P/L	0.7b	0.7b	0.8ab	1.0a	3.66	2.71	
W, $x10^{-4}$J	130a	96b	88b	87b	11.63*	5.24*	
Creep							
Max. strain, %	16.1a	15.7a	12.4b	11.9b	25.32**	92.88**	5.39*
Oscillatory[f]							
G', Pa	9386b	9388b	10206a	9860ab	4.84*	58.49**	1.59
Tan δ	0.51a	0.50b	0.47c	0.46d	280.01**	32.50**	14.50**

[a] Values followed by the same letter are not significantly different.
[b] Insufficient sample prevented replication of farinograph and alveograph tests, therefore interaction terms could not be calculated.
[c] Abs. = % water absorption; DDT = dough development time
[d] MT mixing time; PDR peak dough resistance; MS mixing stability; WI work input.
[e] L= length of curve; P/L = peak height/curve length; W = work of deformation until rupture
[f] G' storage modulus; tan δ shear loss modulus/storage modulus.
** Significant at $p<0.001$
* Significant at $p<0.05$

Tan δ was strongly affected by both protein content and environment ($p<0.001$), and there was a significant interaction between the two factors ($p<0.001$). Tan δ increase was strongly correlated with increasing protein content ($r=0.93$), with each protein level

being significantly different from the others (Table 3.1). As mentioned above, gliadin content has been seen to accumulate as durum protein content increases (Dexter et al. 1989), concomitant with increased alveograph extensibility (Dexter et al. 1994). Therefore, the increase in tan δ with increasing protein content was predictable.

Both protein content and environment ($p < 0.001$) significantly affected maximum creep (% strain). Least significant difference separated the two higher protein samples, which were more extensible, from the two lower protein samples (Table 3.1). Alveograph P/L value, a ratio of peak height to extensibility, increased from low and intermediate low to high and intermediate high protein content (Table 3.1), indicating increased extensibility as protein content increased. Composites prepared from the 1997 crop exhibited the highest creep values, and 1995 the lowest. Because there was a pattern of declining creep and increasing G' with age of the samples the possibility of oxidation modifying dough properties during semolina storage cannot be ruled out.

Varietal Effects

North American Durum Varieties

Typical of their very strong gluten, Durex and AC Melita micro-mixograph curves had longer mixing times, higher mixing stabilities and higher work input requirements than

the other three North American varieties (Table 3.2). Stewart 63 had the weakest mixing characteristics with very short mixing time, rapid curve breakdown and low work input. Peak dough resistance (peak height) was strongly affected by semolina protein content. Nevertheless, even at low protein content Durex 2 (11.6%) and AC Melita 2 (10.1%) had stronger mixing curve characteristics (longer mixing time, higher mixing stability and work input) than Kyle 1 and Wascana 1, both of which were significantly higher in protein, 14.1% and 14.9% respectively, indicative of the superior strength of the former two varieties.

Table 3.2 Physical dough properties of North American durum wheat varieties.

	Stewart 63	**Wascana**		**Kyle**		**Durex**		**AC Melita**	
Location[a]		1	2	1	2	1	2	1	2
Protein, %	12.2	14.9	10.0	14.1	9.8	12.9	11.6	14.1	10.1
Farinograph[b]									
Abs. %	56.9	57.1	53.3	56.8	53.7	57.3	57.7	60.4	55.9
DDT, min	3.2	4.3	3.2	3.3	3.7	4.8	5.0	4.2	4.8
Stability, min	2.4	4.2	2.9	5.5	7.2	8.7	12.4	5.3	12.6
2g Mixograph[c,d]									
MT, min	1.7e	2.0d	2.4c	2.5c	3.0b	3.2b	3.7a	3.2b	3.6a
PDR, units	42.8f	54.9d	39.3g	64.5b	43.7f	72.2a	59.1c	71.1a	49.1e
MS, units	10.9ef	12.7de	9.5f	16.3c	14.6cd	28.2a	27.3a	23.2b	22.3b
WI, %torque·min	53d	76c	68cd	109b	82c	148a	148a	151a	124b
Alveograph[e]									
L, mm	60	93	63	108	86	93	63	113	67
P/L	0.6	0.4	0.5	0.4	0.7	0.9	1.7	0.6	1.2
W, x 10^{-4}J	45	72	45	108	98	251	263	214	187

[a] Swift Current = 1, Regina = 2
[b] Abs. % water absorption; DDT dough development time
[c] Values followed by the same letter are not significantly different
[d] MT mixing time; PDR peak dough resistance; MS mixing stability; WI work input
[e] L curve length; P/L peak height/curve length; W work of deformation to rupture

North American durum wheat varieties were generally extensible, with low, long alveograph curves (Table 3.2). Most had P/L ratios of less than 1. Extensibility was to

some degree related to protein content, with the lower protein replicates having lower L values and higher P/L ratios than their higher protein counterparts. Differences in optimum water absorption requirements, indicated by farinograph (Table 3.2), were not sufficient to account for alveograph dough extensibility differences. Durex and AC Melita had the strongest curves according to W values (range 187 x 10^{-4} to 263 x 10^{-4} J). Stewart 63 was weakest of the varieties with W value of 45 x 10^{-4}J.

Although there were significant differences in G' among samples ($p<0.001$), the only variety to show a significant difference at different locations was Wascana (Table 3.3). Durex and AC Melita samples were not significantly different from one another and exhibited the highest G' values. Stewart 63 had the lowest G', as was expected given its poor performance in alveograph, farinograph and micro-mixograph testing. Wascana and Kyle had intermediate G' values. There was strong correlation between G' and micro-mixograph mixing time (MT) ($r=0.86$), mixing stability (MS) ($r=-0.80$) and work input (WI) ($r=0.84$). Alveograph P/L and W were also strongly related to G' ($r=0.93$ and 0.78, respectively).

The lack of difference in G' between variety replicates was of particular interest because there were large differences in protein content between growing locations. For example, there was a 4% difference in protein content between samples of AC Melita, yet their G' values were not significantly different from each other. This tends to indicate that values obtained were generally indicative of intrinsic gluten quality characteristics of a particular variety, and were not a function of protein content.

Table 3.3. Dynamic Oscillatory Measurements of North American Durum Wheat Varieties[a] Using 0.5% Strain. Data taken at 2 Hz.

Wheat Varieties	Protein (%)	G' (Pa)	Tan δ
Stewart 63	12.2	7175d	0.52a
Wascana 1	14.9	8813b	0.53a
Wascana 2	10.0	7844cd	0.48bc
Kyle 1	14.1	7965bcd	0.52a
Kyle 2	9.8	8544bc	0.48cd
Durex 1	12.9	10087a	0.47cd
Durex 2	11.6	10573a	0.48bc
AC Melita 1	14.1	10178a	0.49b
AC Melita 2	10.1	10188a	0.46d

[a] Values followed by the same letter are not significantly different ($p>0.05$)

Variety had a significant effect ($p<0.001$) on tan δ values (Table 3.3). However, when tested by LSD, varieties segregated by protein content rather than by protein strength as measured by farinograph, alveograph or micro-mixograph. Tan δ tended to decrease as dough protein content decreased; the same trend that was demonstrated for the commercial CWAD composites varying in protein content (Table 3.1). It appeared that tan δ was not an effective parameter for evaluation of durum semolina dough strength when assessing samples of varied environmental origin due to the strong influence of protein content.

The large deformation creep tests were successful at ranking varieties according to their expected level of dough strength as measured by alveograph (Table 3.2 and Fig. 3.6). Differences between varieties in maximum % strain attained during creep were

significantly different ($p<0.001$). Stewart 63 was the weakest, most extensible variety reaching 26.1% strain during creep. Durex and AC Melita were the most resistant to deformation, and were not significantly different from each other, nor were there differences between environments. The difference in protein content between locations was as much as 4% and Stewart 63 had a protein content of 12.2%, which was lower than all of those grown at location 1. One would expect, based solely on protein content as seen with the CWAD protein composites, that the higher protein samples of Wascana, Kyle, Durex and AC Melita from location 1 should have been more extensible than Stewart 63. This, however, was not the case. Therefore, the greater maximum creep of Stewart 63 could not be accounted for by protein content, so logically must reflect reduced resistance to deformation due to weaker gluten.

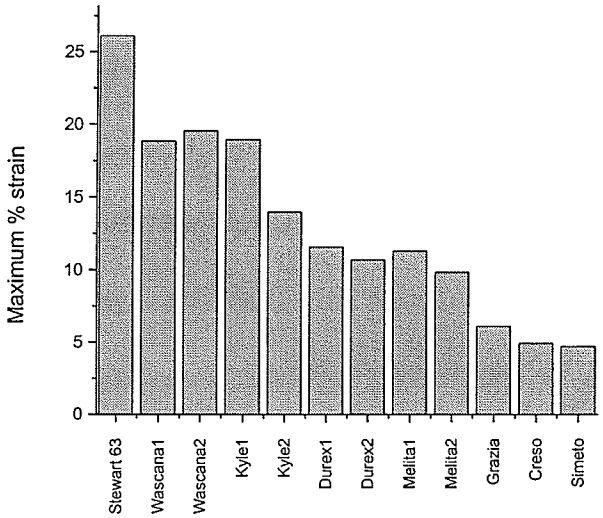

Figure 3.6. Maximum % strain of durum wheat varieties as measured using a creep-recovery test, in shear mode, applying 100 Pa stress for 300 s.

The two samples of Wascana were not significantly different from each other, and were intermediate in extensibility between Stewart 63 and values for Durex and AC Melita. The samples of Kyle were different from each other. Kyle 1 (14.1% protein) was ranked similar to Wascana, whereas Kyle 2 (9.8% protein) was less extensible than Kyle 1, but more extensible than either Durex or AC Melita. The reduced extensibility of Kyle 2 was consistent with its low protein content. As noted earlier, commercial CWAD samples of high and intermediate high protein exhibited greater maximum creep than low and intermediate low protein samples. The commercial samples were made up predominantly of the variety Kyle, therefore, the large reduction in extensibility with very low protein may be characteristic of the variety.

Creep maximum % strain was strongly negatively correlated with micro-mixograph MT ($r=-0.97$), MS ($r=-0.83$) and WI ($r=-0.96$), as well as with alveograph P/L ($r=-0.77$) and W ($r=0.90$).

Italian Durum Varieties

Italian durum wheat lines Creso, Grazia and Simeto were characterized by strong, inextensible gluten properties which resulted in very high, short alveograph curves, with P/L ratios ranging from 1.6 to 3.5 (Table 3.4). Micro-mixograph data (Table 3.4) also indicated strong gluten characteristics, with longer mixing time and higher work input than the North American varieties, even though all three samples were relatively low in protein (9.7% to 11.1%). They made an ideal series to determine if our testing protocols were reflective of intrinsic gluten quality characteristics because they exhibited such

different rheological characteristics, but had similar protein content to several of the North American lines.

Table 3.4. Italian durum wheat physical dough and dynamic rheological properties.

	Creso	Grazia	Simeto
Protein, %	9.7	11.1	10.0
Farinograph			
Absorption, %	57.4	58.0	57.4
DDT[a], min	3.3	4.0	2.8
Stability, min	7.3	6.6	8.5
Micro-Mixograph[b]			
MT, min	4.4a	3.9b	4.1ab
PDR, units	50.6b	62.0a	58.5a
MS, units	21.9b	29.3a	27.2ab
WI, %torque.min	174a	163a	169a
Alveograph[c]			
P/L	3.5	1.6	2.6
W, $x10^{-4}$ J	245	275	231
Oscillatory[d]			
G', Pa	16204a	13695b	16300a
tan δ	0.43a	0.43a	0.42b

[a] DDT dough development time.
[b] MT mixing time; PDR peak dough resistance; MS mixing stability; WI work input.
[c] P/L peak height/curve length; W work of deformation to rupture.
[d] G' storage modulus; tan δ shear loss modulus/storage modulus

All three Italian varieties had much higher G' values and lower tan δ than any of the North American varieties analyzed, consistent with their alveograph and micro-mixograph results (Table 3.4). Maximum % strain also was very low, ranging from 4.7% to 6.1%, compared to 9.8% to 26.1% for the North American varieties (Fig. 3.6). Grazia exhibited the greatest extensibility of the three Italian varieties by alveograph,

dynamic oscillatory and creep measurements. Reduced extensibility of the Italian lines, relative to the North American lines, resulting in low creep, high G' and low tan δ for the former, could not be explained exclusively by low protein content. The effect of environment cannot be ruled out as a contributor to the qualitative differences between the Italian and North American lines. However, the three Italian samples came from different regions of Italy, and their similarity in rheological properties to each other suggests that they are intrinsically different from North American varieties.

Conclusions

Tandem use of the 2g micro-mixograph and a dynamic rheometer provided an excellent means of assessing dough strength of durum semolina and should prove useful, particularly where sample size is limiting, such as in reconstitution experiments. Dynamic mechanical measurements were able to segregate samples according to dough strength as measured by the alveograph while using far less sample. Alveograph P/L ratio and W value were strongly correlated with G' (r=0.93, 0.78), and with large deformation creep maximum % strain (r=-0.77, -0.90).

Intrinsic dough strength could be established either by measurement of G' or by maximum creep. Tan δ was strongly influenced by protein content. Therefore, ideally comparisons among samples of durum wheat semolina should be made on samples grown under similar environmental conditions. The large deformation creep method had the advantage that one could discern very quickly from the maximum height of the

curve (% strain), without any further calculations, the relative strength and extensibility of dough samples.

CHAPTER 4

Viscoelastic Properties of Durum and Common Wheat Dough of Different Strength[1]

ABSTRACT

Linear viscoelastic properties (LVP) were determined for five durum wheat doughs and five common wheat doughs (representing four different classes of Canadian common wheat) of different strength using creep testing. A creep time of 10,000 s was sufficient to reach a state of steady state flow for all of the doughs. Creep compliances were analyzed in terms of a Burgers model. For the durum doughs, the entire elastic compliance curve was shifted to higher values as the strength of the dough (as measured by extensigraph) decreased, while the steady state viscosity increased with strength. For common wheat doughs, the elastic compliance curves were steeper and the steady state viscosities were lower than for durum doughs of comparable extensigraph strength. The retardation strengths associated with a maximum in the retardation spectra were lower for the stronger durum doughs than for common wheat doughs of comparable strength. Differences in the LVP between durum and common wheat doughs of similar extensigraph strength were interpreted in the context of physical gels with crosslinks and entanglements, whose contributions to material properties are difficult to distinguish in short-time creep or dynamic measurements. The increased extensibility of common wheat doughs relative to durum doughs of comparable extensigraph strength was attributed to a higher molecular weight fraction in the polypeptide chains, similar in

some respects to end-linked bimodal polymer networks. The idea of considering these doughs as physical gels was supported by their stress relaxation behaviour.

[1]Published in Rheologica Acta (2001) 40:142-153. By N.M. Edwards, D. Peressini, J.E. Dexter and S.J. Mulvaney.

INTRODUCTION

Baked goods and pasta products are generally produced from different classes and cultivars of wheat flours. Common wheats (*Triticum aestivum*) are preferred for white pan bread, Asian noodles and cakes and pastries. Breeding targets for common wheat intended for high volume pan bread include striking a balance between elasticity and extensibility to ensure good sheeting properties, and an ability to expand and hold gas during the baking process (Dexter 1993). For Asian noodles intermediate strength is preferred, with an emphasis on extensibility for optimum sheeting properties. For confectionery products, a weak gluten is best to impart the desired textural attributes.

Durum wheats (*Triticum durum*) are used around the world primarily for the manufacture of pasta, which is extruded, and for couscous, which is made by agglomerating semolina (a coarse flour). Extensibility is not required for either process, although strong gluten is recognized as an asset for good pasta cooking quality (Feillet and Dexter 1996). Efforts to breed strong gluten durum wheat lines for improved pasta texture have resulted in tenacious inextensible gluten (Quaglia 1988, Rao et al. 2001). Durum wheat is increasingly being used for flat breads and specialty breads, primarily in Mediterranean countries. There is interest in developing strong cultivars with a better balance between resistance to extension and extensibility for more general end-use application (Liu et al. 1996).

Genetically, durum wheats are tetraploids (AABB), and are lacking the D genome found in hexaploid common wheats (AABBDD). The D genome is believed to code for the very high molecular weight glutenins that give bread wheats their superior bread making qualities. Redaelli and coworkers (1997), working with common wheat near-isogenic lines, demonstrated that chromosome 1D strongly influenced both dough elasticity and extensibility. Removal of the D genome from hexaploid bread wheat greatly reduces its baking potential (Kerber and Tipples 1969) and the absence of the D genome is considered at least partly responsible for the relatively poor bread making quality of durum wheat.

There is an abundance of experimental evidence indicating that both wheat flour doughs and their respective glutens are viscoelastic. This literature has been summarized in a number of reviews from different perspectives (e.g., Baird and Labropoulos 1982, Eliasson, 1990, Faubion and Hoseney 1990). Baird and Labropoulos (1982) focused on those studies leading to well-defined material functions and application of viscoelastic models to food doughs in general. They noted that food doughs exhibit both storage and loss moduli in small amplitude oscillatory testing, stress overshoot at the start up of shear flow, stress relaxation at the cessation of flow, viscosity which depends on shear rate, and normal stress differences. However, they also noted that the chemistry of food systems was much more complicated than for polymeric systems such as polymer melts in which the viscoelastic properties are attributed to an entanglement network. Even so, attempts have been made to apply constitutive models to doughs and glutens. Dus and Kokini (1990) have modeled the nonlinear viscoelastic properties of a hard wheat

dough, and Wang and Kokini (1995) the same for a commercially available hydrated gluten using the Bird-Carreau model. Although agreement between the model and experimental data was good for the wheat dough and gluten, it must be pointed out that the Bird-Carreau model was developed and applied to dilute polymer solutions and polymer melts. Thus, the empirical constants of the Bird-Carreau model obtained for wheat doughs and glutens, which do show evidence of entanglement plateaux in stress relaxation data (e.g., Bohlin and Carlson 1981, Rao et al. 2000), probably cannot be related to the same molecular theories of linear viscoelasticity as was originally done. As will be discussed below, the fact that doughs and gluten are gel-like materials at rest, but show extensive creep flow and stress relaxation at long times suggests that wheat doughs and glutens should be considered as physical gels containing both reversible crosslinks and entanglements.

Unlike synthetic polymers, which are generally characterized by their molecular properties (e.g., molecular weight, polydispersity index, zero shear viscosity), individual wheat cultivars are classified by cereal scientists according to their strength. Strength is related to the mixing properties of a particular cultivar and the resistance to extension of the dough after mixing. Generally, as the strength of a cultivar increases the dough development time increases, and its subsequent resistance to extension also increases. However, strength in this context does not have the same precise meaning that it does in materials science, e.g., stress at rupture in a tensile test. Strength in the cereal science sense is probably an attempt to capture in a single concept the blend of strength, extensibility and toughness that a dough exhibits. Although the strength of mixed

doughs clearly originates from its gluten, the actual molecular structure of the hydrated gluten polymer is still a matter of debate.

Gluten has been characterized as having a bimodal distribution between gliadin proteins, where subunits are not joined by disulfide linkages, and polymeric glutenin proteins (Wrigley and Bekes 1999). However, the glutenin fraction itself is also polydisperse. Hydrated gliadin alone is viscous, while hydrated glutenin is a tough, rubbery material. These phenomena have sometimes led to the erroneous assumption in the cereal science literature that gliadin provides the viscous component of gluten, while glutenin is only responsible for its elastic properties. In part this may be due to the extensive use of large strain, empirical instrumental methods to determine the physical properties of doughs (i.e., their strength) rather than characterization of their strength in terms of their fundamental linear viscoelastic properties (LVP). For polymer systems of very high molecular weight, determination of the LVP is increasingly being viewed as a means of molecular characterization due to the difficulties in solubilizing and separating very large polymers using traditional gel permeation chromatography or SE-HPLC (Marin and Montfort 1996). Applying some of these same rheological approaches to wheat doughs of different strength may help to better understand the underlying macromolecular structures in gluten that are responsible for its unique physical properties.

The actual macromolecular structure of the glutenin polymer is still not known for certain. The varied protein-protein interactions in gluten and their possible relationships

to dough rheology have been summarized very well by Wrigley et al. (1998). However, it is still worth interpreting some of the earlier hypotheses for glutenin structure in a modern polymer science context. Two hypotheses, one proposed by Greenwood and Ewart (1975) and the other by Bloksma (1975), together seem to capture the essence of what the structure of glutenin polymer might be. Greenwood and Ewart (1974) proposed that the entangled long, linear (unbranched) molecules thought to be needed for rubberlike elasticity were formed in glutenin during dough mixing by the end-to-end linking ("concatenation" in the author's words) of individual polypeptides via disulfide (S-S) bonds. The rubberlike elasticity of glutenin was supposedly due to entanglements and other secondary interactions, but polymerization of polypeptide units by S-S bonds was a prerequisite to entanglements. Bloksma (1975) proposed that the elastic deformation of dough at a particular stress was related to the number of rheologically effective disulfide cross-links, and that viscous flow was the result of breaking and reforming of these disulfide bonds via a thiol-disulfide interchange reaction. This latter hypothesis is very similar to that for a cross-linked rubber with breaking and reforming of cross-links during deformation as described by Alfrey (1945). Taken together, these two hypotheses actually describe a physical gel consisting of entangled polymer chains and physical crosslinks together. Networks with reversible junctions exhibit highly enhanced viscoelastic properties which are quite different from polymer melts with entanglements (Tanaka and Edwards 1992a).

Furthermore, it is generally assumed that polypeptide chains in the glutenin protein fraction have the potential for forming one or more disulfide bonds only at their ends.

Thus, the number of physical crosslinks would actually increase as the average molecular weight decreased (due to a greater concentration of shorter chain length glutenin subunits) for the same protein content. This suggests that strength in bread wheat and durum doughs may be strongly influenced by lower molecular weight glutenin polypeptides. This is contrary to the conventional wisdom that strength in glutenin is due primarily to entanglements and/or the molecular weight distribution in the highest molecular weight fraction only (Weegels at al. 1996), which assumes gluten is similar to an uncrosslinked polymer melt.

Relatively few studies have determined the linear viscoelastic behaviour of doughs or glutens in creep or stress relaxation as it relates to differences in strength, especially differences between durum and common wheat doughs. None appear to include interpretation of the experimental results in the context of physical gels. For a physical gel, the LVP will clearly depend upon both the number of crosslinks and entanglements, and the time scale of measurement relative to the average lifetime of the crosslinks and the terminal relaxation time of the physical gel (Leibler et al. 1991). Previous experimental creep results have indicated that gluten gel can be considered as ideal elastic at shorter times, and viscous at longer times, with an intervening period of retarded elasticity (Funt Bar-David and Lerchenthal 1975, Hibberd and Parker 1979). Gluten gel has also been found to relax stress completely in about 10,000 s (Bohlin and Carlson 1981, Funt Bar-David and Lerchenthal 1975). All of these results are consistent with a physical gel containing physical crosslinks and entanglements. Clearly, both crosslinks and entanglements could contribute to short time or high frequency small

amplitude oscillatory measurements of dough strength, or high rate of deformation large-strain rheological assessments of dough strength. Physical crosslinks would also be expected to increase the longest relaxation times of the physical gel and increase its viscosity (Liebler et al. 1991).

The objectives of this work were to determine the LVP of doughs from common wheat and durum wheat cultivars representing a broad range of strength and of known end product quality, using creep testing. Creep testing was chosen because it is assumed that the blend of elasticity and viscosity of dough is somehow critical to their functional performance in processing. However, there is very little published data comparing the dynamic and/or steady state elastic compliances or zero shear viscosities of doughs of different strength. In addition, this work will be supported by size exclusion high performance liquid chromatography (SE-HPLC) analysis of the relative amounts and distributions of the glutenin and gliadin protein fractions, large strain (nonlinear) descriptive rheological properties, and baking performance of each cultivar. Recent work characterizing some of these same doughs in stress relaxation showed that stronger doughs were characterized by longer relaxation times and more extensive entanglement plateaux (Rao et al. 2000, 2001). It was of interest here to determine whether differences in dough strength among and between bread wheat and durum doughs would also be reflected in their retardation times, compliances and viscosities. It is expected that this integrated approach involving the chemistry, descriptive rheology, and LVP (both stress relaxation and creep) of a broad range of wheat cultivars will

result in new insights as to how differences in macromolecular structures in doughs are related to differences in their strength and their bread-making performance.

MATERIALS AND METHODS

Wheat cultivars

Five cultivars of Canada Western Amber Durum (CWAD) wheat with a wide range in gluten strength were chosen for this study. Stewart 63 is an old variety no longer grown commercially, with very weak gluten. Wascana, AC Avonlea and AC Morse are currently registered varieties ranging in gluten strength from relatively weak (Wascana) to moderately strong (AC Morse and AC Avonlea). AC Pathfinder was registered in 1998 for test marketing outside of the CWAD class as a blending wheat, due to its extraordinarily strong gluten.

The five common wheat cultivars chosen represent 4 different classes of Canadian common wheat, all with different targeted end uses and, therefore, different gluten quality characteristics. All were bred and selected for a high degree of dough extensibility, but for different degrees of elasticity. Canada Western Extra Strong (CWES) wheat (breeding line ES 12) is primarily intended as a blending wheat to improve dough strength, so CWES varieties have extraordinarily strong gluten and long mixing requirements. Canada Western Red Spring (CWRS) is a premium bread wheat class for production of high quality bread, with moderate to strong gluten and balanced

dough properties. The varieties Neepawa and Laura represent the minimum and maximum gluten strength for the class, respectively. The Canada Prairie Spring White (CPSW) class (breeding line HY 443) is intended primarily for production of Asian noodles and has more moderate gluten strength and lower protein content than CWRS. Canada Western Soft White Spring (CWSWS) wheat (breeding line SWS 238) is primarily used for production of cakes and cookies, so has intrinsically low protein and weak gluten.

Wheat processing properties

Unlike synthetic polymers or elastomers, it is nearly impossible to characterize the molecular level properties of the gluten (e.g., molecular weight distribution, molecular weight between cross-links, zero shear viscosity, number of effective disulfide bonds etc.) in mixed dough. Therefore, Figures 4.1 and 4.2 and Tables 4.1 and 4.2 represent the analytical composition of the 10 wheat cultivars and doughs used in this study, along with their mixing properties and baking characteristics.

Wheats were milled as previously described (Rao et al. 2000, 2001). Flour and semolina protein contents (%N X 5.7) were determined by Combustion Nitrogen Analysis (LECO Model FP-428 Dumas CNA Analyzer, St. Joseph, MD). Optimum water absorption (Tables 4.1 and 4.2) was determined by farinograph (CW Brabender, South Hackensack, NJ) by AACC (2000) Method 54-21. A 2g direct drive mixograph (National Manufacturing Division, TMCO, Lincoln, NE) was used to further rank

samples according to mixing strength and to determine time required to mix to peak consistency (Figs. 4.1 and 4.2).

Doughs from different wheat cultivars necessarily are prepared at different moisture contents (absorption), due to their inherent differences in water absorption. Extensigraph (CW Brabender, South Hackensack, NJ) tests were performed by the American Association of Cereal Chemists (AACC) (2000) Method 54-10 at farinograph water absorption to establish dough strength. The strength of a wheat cultivar can be quantitatively evaluated from its maximum resistance to extension in Brabender units (BU), extensibility (curve length in mm) and area under the extensigraph curve. Extensigraph curves are shown in Figures 4.1 and 4.2 for each cultivar. Using these criteria, the order of strength of the five durum cultivars (high to low) was (AC Pathfinder > AC Morse > AC Avonlea > Wascana > Stewart 63. These extensigraph rankings related directly to mixograph peak resistance and work input (Fig. 4.1). Similarly, for the common wheat cultivars, the order of strength was ES12 > Laura > Neepawa > HY 443 > SWS 238. Although empirical in nature, the extensigraphs do show large differences in the dough extensional properties for the same experimental conditions (sample geometry and timeframe of deformation).

A long fermentation straight dough (remix-to-peak) baking procedure was used to evaluate baking potential (Kilborn and Tipples 1981). After initial mixing, dough is subjected to a 160 minute initial fermentation, followed by remixing in a recording dough mixer to 10% past peak consistency, sheeting molding and panning, prior to final

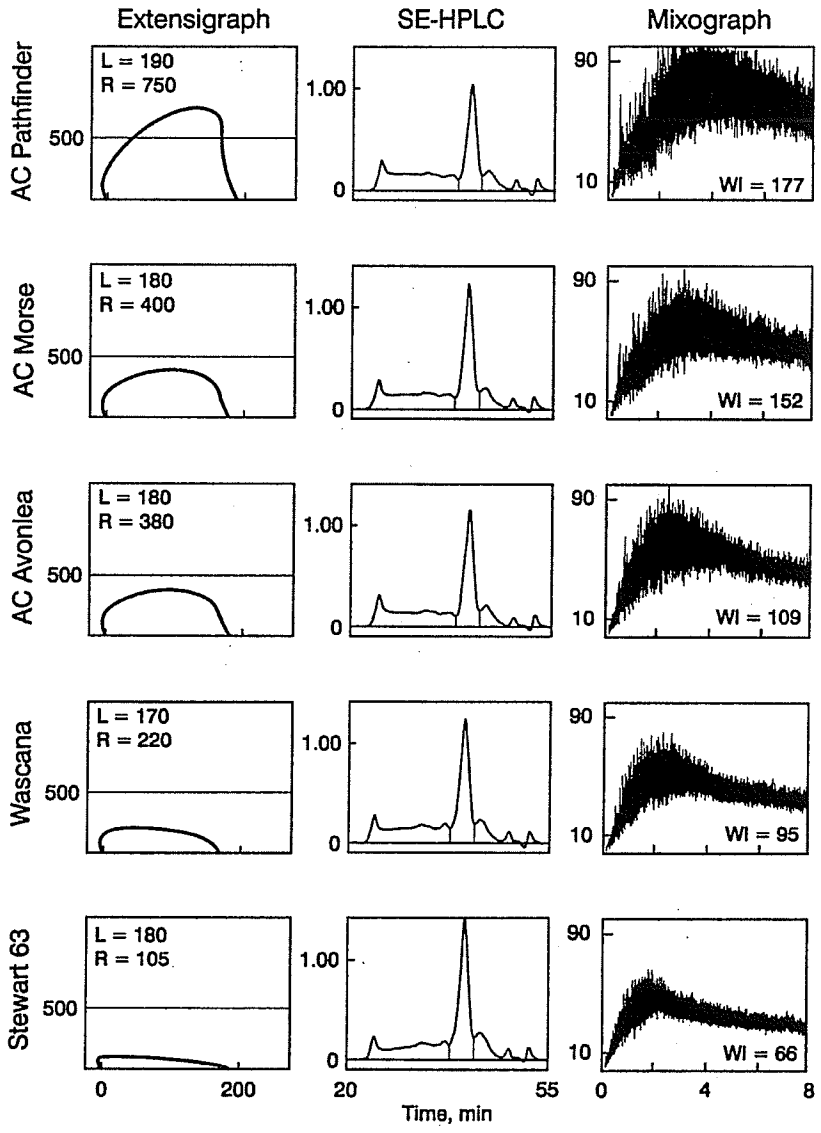

Figure 4.1. Durum wheat semolina extensigrams, SE-HPLC profiles and 2g-mixograms. L = extensibility, mm; R = maximum curve height, Brabender Units (BU); WI = work input to peak, arbitrary units.

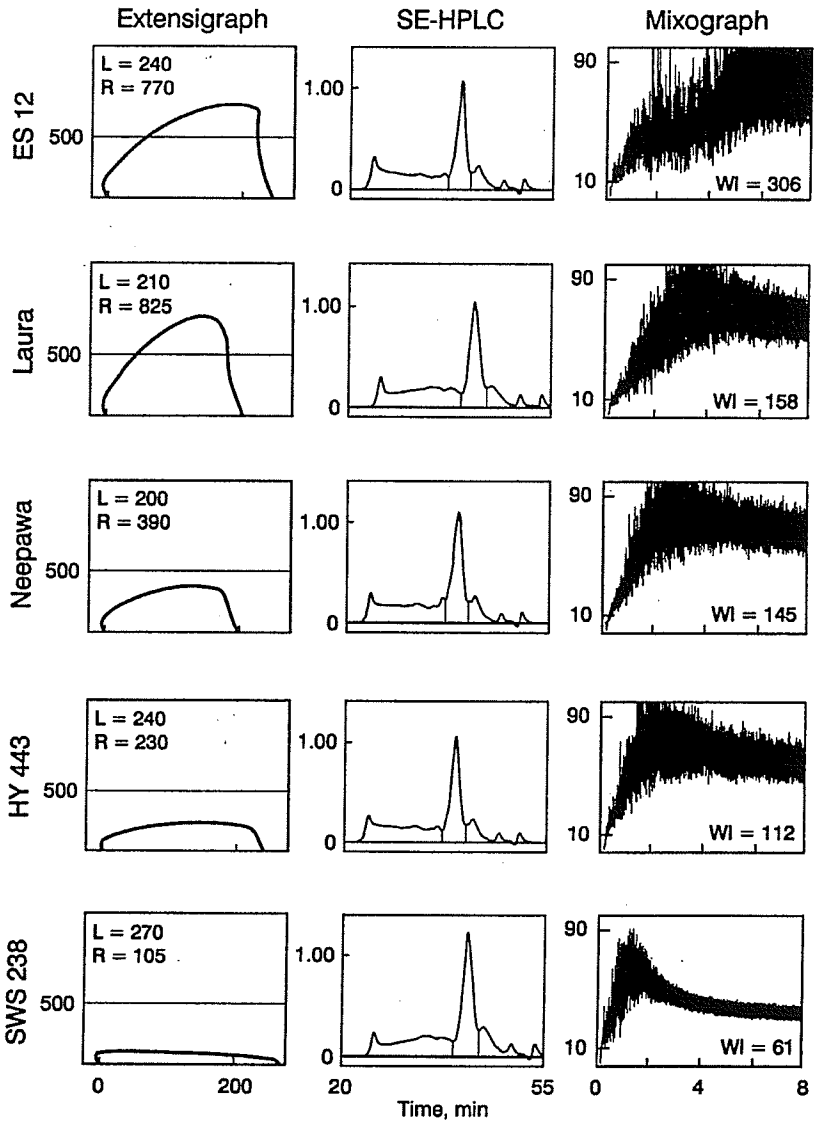

Figure 4.2. Common wheat flour extensigrams, SE-HPLC profiles and 2g-mixograms. L = extensibility, mm; R = maximum curve height, BU; WI = work input to peak, arbitrary units.

proof and baking. Loaf volumes are determined by rapeseed displacement. Baking strength index, a measure of loaf volume potential at a given protein level is determined as described by Tipples and Kilborn (1974).

Table 4.1. Durum wheat semolina quality, dough mixing properties and baking quality

	AC Pathfinder	*AC Morse*	*AC Avonlea*	*Wascana*	*Stewart 63*
Semolina					
Protein, %	11.9	12.0	12.8	12.2	12.0
Farinograph					
Absorption, %	57.8	55.7	55.4	55.0	55.0
2g Mixograph					
Time to Peak, min.	3.6	3.4	2.7	2.6	2.0
Absorption, %	50	50	50	50	50
Dough moisture,%	42.7	42.7	42.7	42.7	42.7
Dough Protein, %	7.9	8.0	8.5	8.1	8.0
SE-HPLC					
Gliadin/Glutenin	0.60	0.73	0.71	0.76	0.89
Unextractable Protein, %	17	16	15	15	16
Remix-to-Peak Bread					
Remix-to-peak					
Energy, Whr/kg	3.3	2.3	2.0	1.3	0.5
Loaf volume, cc	715	610	550	470	390
Baking Strength Index, %	92	78	66	59	50

The breadmaking performance of the durum wheat cultivars, as given by bread loaf volume (Table 4.1) corresponded to the order of strength, with higher strength giving greater loaf volumes, although even the strongest durum had only 90% of the baking potential of a good bread wheat. Among the common wheat cultivars, ES 12, Laura, Neepawa and HY 443 all gave good bread loaf volumes, and BSI values higher than for any durum, although BSI rankings were related to order of strength. The very weak soft wheat cultivar SWS 238 gave a low value for loaf volume (Table 4.2).

Table 4.2. Common wheat flour quality, mixing properties and baking quality

	ES 12	*Laura*	*Neepawa*	*HY 443*	*SWS 238*
Flour Protein, %	12.0	12.3	13.6	11.7	9.8
Farinograph					
Absorption, %	62.9	64.5	65.9	65.1	56.1
2g Mixograph					
Time to Peak, min.	6.1	3.9	3.2	2.8	1.5
Absorption, %	60	60	60	60	50
Dough moisture, %	46.3	46.3	46.3	46.3	42.7
Dough Protein, %	7.5	7.7	8.5	7.3	6.5
SE-HPLC					
Gliadin/Glutenin	0.53	0.67	0.72	0.76	0.60
Unextractable Protein, %	25	13	16	12	11
Remix-to-Peak Bread					
Remix-to-peak					
Energy, Whr/kg	3.6	2.6	2.9	1.9	0.6
Loaf volume, cc	920	925	860	740	390
Baking Strength Index, %	118	115	96	97	54

Size exclusion high performance liquid chromatography (SE-HPLC)

Wheat protein extraction and separation were performed as described by Nightingale et al. (1999). Relative proportions of the extracted major gluten protein fractions were measured by SE-HPLC. Chromatograms are shown in Figs. 4.1 and 4.2. The relative proportions of gliadins and glutenins in protein extracts were derived from areas under the SE-HPLC profiles. The protein content of the pellet remaining after centrifugation following protein extraction was determined by Combustion Nitrogen Analysis. The unextractable protein was considered to represent very high molecular weight glutenin polymeric protein (Weegels et al. 1996), and was included in the glutenin portion for

calculation of the ratios of gliadin to glutenin (Tables 4.1 and 4.2). The presence of specific HMW glutenin subunits in bread wheats (e.g., 5+10) increases the relative proportion of unextractable protein, and is also considered to confer superior physical dough properties and baking characteristics (Hargreaves et al. 1996).

Creep Experiments

Doughs were prepared for creep testing by mixing semolina or flour and water to one minute past peak in the 2g mixograph (Table 4.1). Dough water absorption was adjusted to approximately 5% below farinograph water absorption. Accordingly, durum wheat semolina and SWS flour were prepared at 50% water absorption (on a 14% flour moisture basis) resulting in dough with 42.7% moisture content. The other common wheat flours were prepared at 60% absorption, giving a final dough moisture content of 46.3%. Final protein content of each prepared dough is presented in Tables 4.1 and 4.2.

Each dough was subject to creep for 10,000 s using a Rheometrics SR500 controlled stress rheometer (Rheometrics Scientific, Piscataway, NJ) in shear. The rheometer was fitted with 25 mm serrated parallel plates and was gapped to 2.75 mm. Excess dough was carefully trimmed and the exposed edges coated with silicon grease to prevent surface drying of the sample. In addition, the rheometer was equipped with a humidity chamber to further prevent dough drying. Samples were rested for an additional 15 minutes after loading prior to testing. All experiments were conducted at 25°C at or below the critical stress for each cultivar, which ranged from 10 Pa to 50 Pa and was

based on the agreement or overlap of creep compliance curves obtained at different stresses.

Creep tests were obtained on three separately mixed doughs for each cultivar. The time of testing combined with the number of replicates made recovery experiments impractical. However, this may not be a serious concern for this study as results are primarily comparative in nature. We are interested in determining trends in the LVP with the known strengths of the doughs rather than attempting to predict their recovery behaviour from creep data.

Analysis of creep curves

The length of creep time (10,000 s) was sufficient to reach a state of constant shear rate for the last several thousand seconds of the test. All of the creep curves showed a similar general form, an apparent instantaneous strain followed by a retarded elastic strain and finally steady state creep. Since all of the creep results were obtained in the linear regime, results are presented as the creep compliance J(t). The compliances were modeled (or curve fitted) using a Maxwell body in series with two Kelvin-Voigt bodies, or Burgers model (Fig. 4.3), giving a total of six parameters as shown in Equation 1 below.

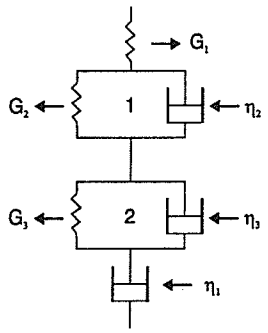

Figure 4.3 Six parameter Burgers model.

$$J(t) = 1/G_1 + 1/G_2\,[1-\exp(-tG_2/\eta_2)] + 1/G_3[1-\exp(-tG_3/\eta_3)] + t/\eta_1 \qquad (1)$$

For convenience, $1/G_1$ actually represents a "short time" compliance (J_1) which lumps together all retardation processes up to 10 s as instantaneous relative to the 10,000 s creep time, $1/G_2\,[1-\exp(-tG_2/\eta_2)] + 1/G_3[1-\exp(-tG_3/\eta_3)]$ will be referred to as retarded elastic compliance (J_2), and t/η_1 will be referred to as viscous flow (J_3), all relative to the creep time of 10,000 s. Dus and Kokini (1990) indicated that the viscosity of a 40% moisture wheat flour dough tended to a zero shear viscosity for shear rates less than 10^{-5} s. Such low steady state shear rates were achieved here.

Model parameters (mean values and S.D.) are shown in Table 4.3 for the durum doughs and Table 4.4 for the common wheat doughs. In addition to the rheological model parameters, the retardation spectrum was also obtained for each dough using a linear least squares analysis software package provided with the rheometer (RSI Orchestrator).

Although empirical in nature, interpretation of the Burgers model parameters and the retardation spectra, in the context of physical gels, provides a powerful heuristic framework for understanding the relationships between macromolecular structures, dough strength and end-product quality.

Table 4.3: Six parameter Burgers model representing creep compliance data (10,000 s) for durum wheat semolina dough. Evaluation performed on average curve from triplicate measurements in the linear viscoelastic range. Mean values ± standard deviation.

Parameters	Stewart 63	Wascana	AC Avonlea	AC Morse	AC Pathfinder
J_1 ($1/G_1$; 1/Pa)	$(5.66 \pm 0.10) \cdot 10^{-4}$	$(4.28 \pm 0.15) \cdot 10^{-4}$	$(3.20 \pm 0.24) \cdot 10^{-4}$	$(2.74 \pm 0.09) \cdot 10^{-4}$	$(2.05 \pm 0.15) \cdot 10^{-4}$
η_1 (Pa s)	$(0.51 \pm 0.01) \cdot 10^{7}$	$(0.86 \pm 0.01) \cdot 10^{7}$	$(0.90 \pm 0.05) \cdot 10^{7}$	$(1.18 \pm 0.04) \cdot 10^{7}$	$(1.98 \pm 0.11) \cdot 10^{7}$
G_2 (Pa)	$(1.12 \pm 0.06) \cdot 10^{3}$	$(1.77 \pm 0.17) \cdot 10^{3}$	$(2.70 \pm 0.34) \cdot 10^{3}$	$(3.05 \pm 0.32) \cdot 10^{3}$	$(5.40 \pm 1.06) \cdot 10^{3}$
η_2 (Pa s)	$(0.32 \pm 0.02) \cdot 10^{6}$	$(0.47 \pm 0.04) \cdot 10^{6}$	$(0.72 \pm 0.08) \cdot 10^{6}$	$(0.83 \pm 0.11) \cdot 10^{6}$	$(1.35 \pm 0.29) \cdot 10^{6}$
G_3 (Pa)	$(0.37 \pm 0.01) \cdot 10^{3}$	$(0.66 \pm 0.05) \cdot 10^{3}$	$(1.01 \pm 0.07) \cdot 10^{3}$	$(1.08 \pm 0.06) \cdot 10^{3}$	$(1.89 \pm 0.12) \cdot 10^{3}$
η_3 (Pa s)	$(0.82 \pm 0.01) \cdot 10^{6}$	$(1.42 \pm 0.11) \cdot 10^{6}$	$(2.21 \pm 0.17) \cdot 10^{6}$	$(2.44 \pm 0.19) \cdot 10^{6}$	$(4.19 \pm 0.40) \cdot 10^{6}$
τ_2 (η_2 / G_2 ; s)	284 ± 4	265 ± 5	266 ± 10	273 ± 13	250 ± 5
τ_3 (η_3 / G_3; s)	2207 ± 46	2175 ± 91	2179 ± 64	2267 ± 143	2213 ± 73

Table 4.4: Six parameter Burgers model representing creep compliance data (10000s) for common wheat flour dough. Evaluation performed on average curve from triplicate measurements in the linear viscoelastic range. Mean values ± standard deviation.

Parameters	SWS 238	HY 443	Neepawa	Laura	ES 12
J_1 ($1/G_1$; 1/Pa)	$(3.50 \pm 0.37) \cdot 10^{-4}$	$(4.50 \pm 0.14) \cdot 10^{-4}$	$(4.57 \pm 0.21) \cdot 10^{-4}$	$(4.51 \pm 0.57) \cdot 10^{-4}$	$(2.83 \pm 0.14) \cdot 10^{-4}$
η_1 (Pa s)	$(6.61 \pm 0.68) \cdot 10^{6}$	$(3.96 \pm 0.19) \cdot 10^{6}$	$(3.54 \pm 0.08) \cdot 10^{6}$	$(3.62 \pm 0.49) \cdot 10^{6}$	$(7.58 \pm 0.20) \cdot 10^{6}$
G_2 (Pa)	$(2.07 \pm 0.37) \cdot 10^{3}$	$(1.56 \pm 0.07) \cdot 10^{3}$	$(1.52 \pm 0.09) \cdot 10^{3}$	$(1.54 \pm 0.26) \cdot 10^{3}$	$(3.09 \pm 0.30) \cdot 10^{3}$
η_2 (Pa s)	$(6.17 \pm 1.44) \cdot 10^{5}$	$(4.97 \pm 0.22) \cdot 10^{5}$	$(4.59 \pm 0.14) \cdot 10^{5}$	$(4.34 \pm 0.79) \cdot 10^{5}$	$(8.63 \pm 0.76) \cdot 10^{5}$
G_3 (Pa)	$(5.91 \pm 0.77) \cdot 10^{2}$	$(4.06 \pm 0.18) \cdot 10^{2}$	$(3.26 \pm 0.02) \cdot 10^{2}$	$(4.21 \pm 0.44) \cdot 10^{2}$	$(7.75 \pm 0.29) \cdot 10^{2}$
η_3 (Pa s)	$(1.48 \pm 0.25) \cdot 10^{6}$	$(1.11 \pm 0.05) \cdot 10^{6}$	$(0.97 \pm 0.02) \cdot 10^{6}$	$(1.16 \pm 0.16) \cdot 10^{6}$	$(2.09 \pm 0.14) \cdot 10^{6}$
τ_2 (η_2 / G_2 ; s)	298 ± 18	319 ± 1	303 ± 8	282 ± 6	280 ± 8
τ_3 (η_3 / G_3; s)	2496 ± 153	2735 ± 68	2975 ± 81	2746 ± 130	2702 ± 94

RESULTS

Figures 4.4a and 4.4b show representative results for the total compliance and the elastic component of the total compliance for the weakest and strongest durum and common wheat doughs, respectively. The elastic compliance was obtained by subtracting the viscous flow component (J_3 of the model) from the experimental creep curve. The model total compliance curve is also plotted with the experimental curves for the durum wheats, and shows very good agreement except at the shortest times. The ratio of J_1 between AC Pathfinder and Stewart 63 was 0.36. Both cultivars then appeared to reach a steady state elastic compliance within the 10,000 s creep time with the ratio between AC Pathfinder's and Stewart's retarded elastic compliances being reduced to 0.20. The steady flow viscosity was nearly four times greater for the AC Pathfinder dough, even though the moisture contents and total protein contents of these two doughs were essentially identical (Table 4.1). However, the ratio of gliadin/glutenin was 0.6 for AC Pathfinder, while it was 0.89 for Stewart 63. It is also clear from the SE-HPLC traces in Figure 4.1 that the distribution of the glutenin fraction is different for the two cultivars. Both show a prominent peak in the HMW region indicating that both cultivars contain at least some amount of the highest molecular weight component. The stronger AC Pathfinder, however, also shows higher amounts of the intermediate higher molecular weight material, while the Stewart 63 molecular weight distribution is skewed more towards the lower molecular weight intermediate materials. The significantly lower value of J_1 for the AC Pathfinder (2.05×10^{-4}) versus Stewart 63 (5.66×10^{-4}) can be reasonably interpreted as indicative of an increase in the number of

initial elastically effective network chains terminated by either physical crosslinks or entanglements. This is consistent with the higher dynamic moduli reported for AC Pathfinder dough relative to Stewart 63 (Rao et al. 2001). The other three durum cultivars of intermediate strength showed qualitatively similar creep behaviour as the AC Pathfinder and Stewart 63 (data not shown). The retarded and elastic compliances for all five durums are shown in Figures 4.5a and 4.6a and will be discussed later together with the common wheat doughs.

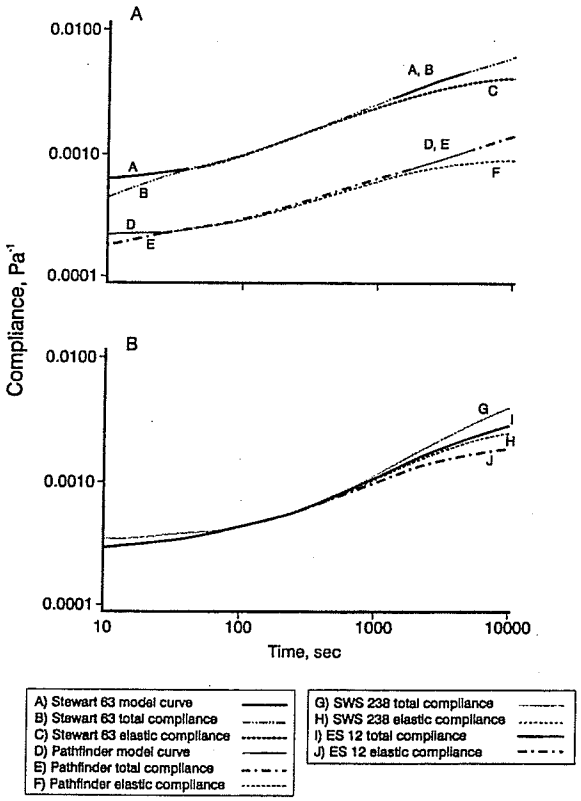

Figure 4.4. Total compliance and elastic compliance ($J(t) - t/\eta_1$) plotted for average of triplicate measurements, and model total compliance from 6 parameter Burgers model. 4.4a. AC Pathfinder and Stewart 63. 4.4b. ES 12 and SWS 238.

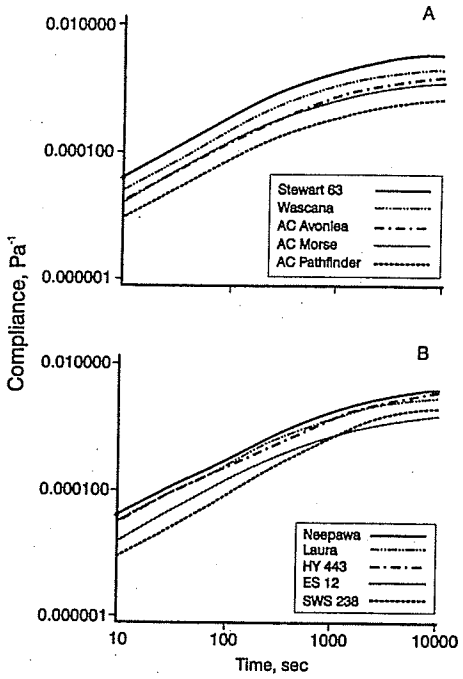

Figure 4.5. Retarded elastic compliance [J(t) - (J_1 + t/η_1)] calculated from model curves derived from 6 parameter Burgers model compliance curves (Tables 4.3 & 4.4). 4.5a. Durum wheat cultivars. 4.5b. Common wheat cultivars.

There are both similarities and differences in the creep behaviour of the doughs for the two common wheat cultivars of very different strength as compared to the durum doughs as shown in Figure 4.4b. Like the durum doughs, both common wheat doughs also show elastic behaviour at short times, but differ at longer times. This result is qualitatively similar to the work of Muller (1969), who found that the elastic compliances of glutens from soft and hard wheats were surprisingly similar, given their large difference in strength. The steady flow viscosities for the common wheat doughs of different strength were much more similar than was found for the durum wheats, being only 1.15 versus the factor of 3.38 found for AC Pathfinder and Stewart 63. The

absolute values of viscosities were also lower for the common wheats than for the durum wheats. Also, the ES 12 dough showed a higher steady state compliance than the AC Pathfinder dough indicating a higher degree of configurational changes of the original network for the ES 12. Unlike the intermediate strength durums, the three intermediate strength common wheat doughs all showed quite similar total compliance curves (data not shown) as indicated by the model parameters in Table 4.4.

As compared to AC Pathfinder and Stewart 63, the large difference in strength for the two common wheat doughs was only evident in their longer-time retardation behaviour. This was reflected in their model τ_3 values: 2702 s for ES 12 versus 2496 s for SWS 238, while τ_3 was essentially identical for the two durums. Also, in general, τ_3 was larger for all of the common wheat doughs relative to the durum doughs, regardless of strength. Overall, it is worth noting that short-time creep testing and/or high frequency dynamic testing of doughs, will apparently not reveal important differences in the long-range configurational and viscous deformations of doughs of different strength.

Elastic compliances

Given the supposed importance of elasticity in doughs, the elastic compliances were also examined separately. Figures 4.5a and 4.5b show the retarded elastic compliances for the five durum and common wheat doughs, respectively. For the durums, it is apparent that all of the curves have a similar shape, consistent with their similar model

retardation times. The trend was for higher elastic compliances throughout the 10,000 s creep time as the ratio of gliadin to glutenin increased at essentially constant dough protein content. This result, combined with the decrease in viscosity as strength decreased, suggests a lower degree of crosslinking as strength decreased. Also, the lower the compliance curve was, the higher was the extensigraph maximum resistance to deformation. This is particularly apparent for the AC Avonlea and AC Morse cultivars, which show only small differences in retarded elasticity over time and differ by only 20 BU in their extensigraph maximum resistance to deformation values. This suggests that not only do these two doughs have similar glutenin contents, but that their distribution of retardation times are also similar, which is consistent with their similar SE-HPLC traces (Figure 4.1). Overall, these results suggest that under a small applied stress, stronger durum doughs show less configurational rearrangement of the original network and less viscous flow, which is consistent with the idea that they are more crosslinked.

For the common wheat doughs, the SWS 238 sample has an obviously different sort of character (distribution of retardation times) than the other common wheats. This could be due to its lower moisture and protein contents relative to the other common wheat doughs, combined with its relatively high proportion of glutenin (Table 4.2). The lower moisture content could explain its stiffness at short times. Looking at the other four common wheat doughs, they show a qualitatively similar behaviour, although the compliance curve for the ES cultivar is clearly shifted to lower values than the other three cultivars. This could be explained by its lower gliadin to glutenin ratio (0.53),

relative to Laura, Neepawa and HY 443, whose gliadin to glutenin ratios were higher and very similar to each other. In fact, their J_1 values are identical (Table 4.1), and the other model parameters are also close to each other. Unlike for the durum doughs of different strength, the three moderately strong (MS) common wheat doughs show very different extensigraph strengths for similar retarded elastic compliance curves and similar ratios of gliadin to glutenin.

Figures 4.6a and 4.6b show the time course of the total elastic compliances (J_1 and J_2) for the durum and common wheat doughs, respectively. It is apparent that the strong and moderately strong durum doughs all show flatter trajectories to their steady state elastic compliance values than the extra strong and moderately strong common wheats. For example, after 1000 s of creep the Neepawa, Laura and HY 443 common wheat doughs all have compliances well above 0.001 Pa^{-1}, while the extra strong ES12 is just about at 0.001 Pa^{-1}. For the durum doughs, the moderately strong AC Avonlea and AC Morse are just approaching 0.001 Pa^{-1}, while the strong AC Pathfinder dough is well below this value. The weak Wascana and very weak Stewart 63 durum doughs are somewhat similar to the moderately strong bread wheat doughs, although their extensigraph strengths and bread loaf volumes are significantly lower than for the bread wheat doughs.

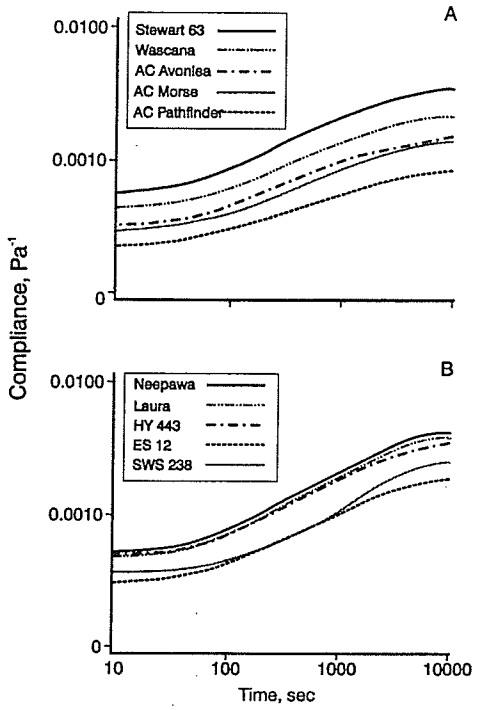

Figure 4.6. Elastic compliance $(J(t) - t/\eta_1)$ of individual wheat lines calculated from model compliance curves. 4.6a. Durum wheat semolina doughs. 4.6b. Common wheat flour doughs.

Retardation Spectra

Subtle differences in the distribution of retardation times are easier to see in the retardation spectra than in the parameters of the mechanical model. The retardation spectra represent a continuous distribution of retardation times and their intensity in a particular timeframe, while the mechanical model gives only average retardation times over a larger time frame. Retardation spectra for all of the doughs are shown in Figures

4.7a and 4.7b on the same scale for the durum and common wheats, respectively. Although the curves are not very smooth they do illustrate the retardation spectra are composed of two distinct processes widely separated in time. This is consistent with the well-known two-stage stress relaxation behaviour exhibited by both doughs and gluten (Bohlin and Carlson 1981). Differences between the durum doughs and common wheat

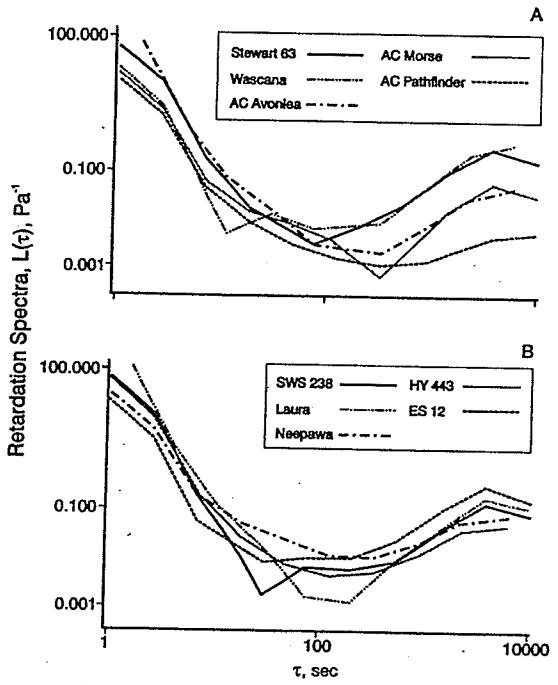

Figure 4.7. Retardation spectra, L(τ), for individual wheat lines calculated by least squares analysis of compliance data collected over 10,000s. 4.7a. Durum wheat semolina doughs. 4.7b. Common wheat flour doughs.

doughs are clearly most evident for the longer retardation times. For the common wheat doughs (excepting SWS 238), higher extensigraph strengths are associated with higher retardation strengths for the maximum in the retardation spectrum, which also leads to

higher viscosities as indicated in Table 4.4. Surprisingly, just the opposite trend is found for the durum doughs. Stronger durum doughs show lower retardation strengths for the longest retardation times, but also higher viscosities. This anomalous effect for the durum doughs could be due to the stronger influence of physical crosslinks on their LVP (higher steady state viscosities for lower molecular weights) as discussed in the Introduction, while common wheat doughs appear to be more similar to uncrosslinked melts with entanglements in the longer time frames.

DISCUSSION

It should be appreciated that an unequivocal connection between rheological measurements and underlying molecular structures cannot be made for these gluten doughs. The chemistry of the gluten complex within a dough is simply too complex. Also, these doughs vary in concentration of protein and glutenin, as well as molecular weight distribution within the glutenin fraction. However, these results have shown that the LVP are sensitive to apparent differences in these underlying macromolecular structures, which in turn appear to be related to the concept of dough strength. The higher overall viscosities of the durum doughs, the greater sensitivity of the viscosity to the extent of plasticization by gliadin and their inextensibility relative to the common wheat doughs suggests strength in the durum doughs is related to the number of physical crosslinks in the glutenin fraction. This is consistent with the concepts of physical gels where a high concentration of lower molecular weight chains with functional crosslinking groups can show unusually high viscosities. However, those

concepts are limited to the LVP. The main purpose of this work was to integrate these LVP with the large strain results (Figures 4.1 and 4.2) and the chemical and composition data (Tables 4.1 and 4.2) to better understand the relationships between the macromolecular structures in doughs, their large strain strength and end-product quality.

Gluten doughs as multimodal networks

If one were to assume that the numerous physical crosslinks in gluten were permanent rather than physical in nature, then there would be an obvious analogy between gluten and polydimethylsiloxane (PDMS) bimodal networks. In short, bimodal PDMS networks containing very short chains end-linked with relatively long chains have unusually large ultimate strengths considering their large extensibilities (Wang and Mark 1992). As described in the Introduction and in many cereal science textbooks, the strength of various wheat cultivars has been linked to their glutens, particularly their HMW glutenin fraction, but not necessarily to their distribution of chain lengths within this fraction. Also, focusing only on the highest molecular weight glutenin fraction neglects the contributions shorter polypeptides can make to strength via their crosslinks, without necessarily decreasing extensibility.

There has also been discussion of the molecular nature of this phenomenon in the literature. Andrady et al. (1980) refer to the fact that fewer short chains in a bimodal network permits a more extensive reapportioning of the strain within the network, with a corresponding increase in extensibility. Smith et al. (1990) showed that the tensile

strength of bimodal PDMS as determined at an extension rate of about 0.3 min^{-1} showed a maximum at about 30-40 weight % short chains. These authors explain the relatively high elongations of these crosslinked networks as being due to the nonaffine nature of the deformation, that is the longer chains in the connected network deform proportionately more than the short chains. However, these long chains must also move through the matrix of shorter chains as they are extended, thus resulting in substantial viscous dissipation (and higher strength) than for the long chains alone. Thus, the higher extensibilities of strong bread wheat doughs generally, could be due to a lower proportion of shorter, crosslinked polypeptides.

For the common wheat doughs, there were large variations in extensigraph strength even though the retardation spectra for the doughs were fairly similar, relative to the durum doughs. Differences in strength for these doughs could be due to fundamentally different distributions of polypeptide chains (and their interactions) than for the durums. It is possible that strength in bread wheat doughs is more related to differences in entanglement networks as suggested by the stress relaxation results of Rao et al. (2000), and that these differences become more evident in the extensigraph which presumably has a much higher effective rate of strain than the creep tests. In the shorter experimental time frame of the extensigraph it may not be possible to distinguish the contributions of entanglements and physical crosslinks to dough strength. Similarly, they may not be distinguished in high frequency dynamic measurements, even though both entanglements and physical crosslinks may contribute to elastic moduli. However, differences in macromolecular structures between the bread wheats and durum doughs

are suggested by their behaviour at longer creep times.

The basic idea of the bimodal networks as expressed by Smith et al. (1990) is that the high tensile strengths are probably due to the low mobility of the chains resulting in high dissipation of energy, which delays rupture. This general concept can be tested by looking at some results for the rate of stress relaxation for some of the doughs discussed above.

Stress relaxation

The stress relaxation behaviour of some of these same doughs has previously been determined (Rao et al. 2000, 2001). The relaxation times should be an indication of their relative rates of molecular motions in dissipating stress. For convenience the initial modulus (Pa) in stress relaxation (G_o) and the time in seconds to relax 75% of the initial stress (t_{75}) will be used for comparison. The values for G_o and t_{75} (G_o ; t_{75}) for the durums were (12,750; 50.1), (6,753; 20.0), (6,507; 20.0), (6,587; 11.2) and (6,087; 8.4) for the AC Pathfinder, AC Morse, AC Avonlea, Wascana and Stewart 63, respectively. There is a clear trend for the extensigraph strength to be related more to the relaxation times than to the initial modulus in stress relaxation. This would tend to support the idea expressed here that strength of the doughs is somehow related to their relative molecular mobilities. It is also interesting to note that AC Avonlea and AC Morse showed essentially identical stress relaxation responses, just as they did here in creep and the extensigraph.

Similar stress relaxation data are available for only three of the common wheat doughs. Values of G_o and t_{75} were (13,040; 50.1); (5,658; 13.4); and (5,213; 5.0), for ES 12, Neepawa and HY 443, respectively. It is quite clear that the extensigraph strength of the common wheat doughs also appears to be related to their relative rates of relaxation. In particular, the strength of the Neepawa (390 BU) was significantly higher than for the HY 443 (230 BU), and this may be reflected in the significantly greater value of t_{75} for the Neepawa dough. In addition, the relaxation times were identical for the AC Pathfinder durum dough as for the ES 12 bread wheat dough and their extensigraph maximum resistance to deformation were also very close, 750 BU and 770 BU, respectively. However, the ES 12 dough was more extensible and showed a higher loaf volume. Neepawa and HY 443 doughs showed shorter relaxation times (13.4 and 5.0 s, respectively) than durum doughs of comparable strength and ratio of gliadin to glutenin (20 s for AC Avonlea and 8.4 s for Wascana, respectively), and also had higher loaf volumes. As discussed above, the three bread wheat doughs also showed higher creep compliances than for durum doughs of similar strength. The trend for longer relaxation times for the durum doughs of comparable strength coupled with their known lack of the D-genome coding for high molecular weight polypeptides further supports the view that the material properties of durum doughs are influenced more by physical crosslinks than are the common wheat doughs.

CONCLUSIONS

A unique aspect of this work is the interpretation of the creep behaviour of doughs of different strength in the context of physical gels containing physical crosslinks and/or entanglements. The overall higher steady state viscosities of the durum doughs and their relative inextensibility suggests that strength in the durum doughs is primarily a function of the density of physical crosslinks present. This is consistent with their lower creep compliances relative to common wheat doughs of similar strength, but higher steady state viscosities. The increase in creep compliance and steady state viscosity for stronger common wheat doughs suggests a greater role of the larger polypeptides in determining the strength of bread wheat doughs. It is pointed out that it may be difficult to distinguish between physical crosslinks and entanglements at short time in creep or dynamic tests, but distinct differences between the durum and bread wheat doughs were observed at longer times in creep and stress relaxation. This could help explain the observation of Ammar et al. (2000) that part of the large strain deformation energy for durum doughs did not contribute to improving baking performance. A similar result was found here where durum doughs gave lower bread loaf volumes than for bread wheat doughs with similar extensigraph maximum resistance to deformation.

By analogy to published literature regarding bimodal PDMS networks, the results presented and discussed here for durum and common wheat doughs suggest that in either case, the strength of these doughs was fundamentally related to their underlying molecular mobilities. This is particularly evident in their respective rates of stress

relaxation, where slower relaxation processes were associated with higher strength for both durum and common wheat doughs. However, longer relaxation times most likely represent different molecular processes in durum and bread wheat doughs.

The idea presented here that the relative balance between strength and extensibility in wheat flour doughs depends upon the distribution of short chains to long chains in their crosslinked and/or entanglement network structures needs to be tested further. Along the lines of the PDMS networks, closer examination of the molecular weight distribution and extent of crosslinking in the glutenin fraction is needed. It would also be helpful if an estimate of the average lifetimes of the various physical crosslinks in doughs could be determined.

CHAPTER 5

The Role of Gluten and Its Components in Determining Durum Wheat Dough Viscoelastic Properties

Abstract

Gluten was isolated from three durum wheat cultivars with a range in strength. Gluten was further fractionated to yield gliadin, glutenin and high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits (GS). The gluten and various fractions were used to enrich a base semolina. Enriched dough samples were prepared at a fixed protein content using a 2g micro-mixograph. Mixing strength, dynamic and creep compliance responses of doughs enriched with added gluten ranked in order according to the strength of the gluten source. Gliadin addition to dough resulted in weaker mixing curves. Gliadin was not able to form a network structure, having essentially no effect on dough compliance, but it did demonstrate its contribution to the viscous nature of dough by increasing tan δ. Source of the gliadin made no difference in response of moduli or compliance. Addition of glutenin to the base semolina increased the overall dough strength properties. Glutenin source did influence both dynamic and compliance results, indicating there were qualitative differences in glutenin among the three cultivars. Enrichment with both HMW-GS and LMW-GS increased overall dough strength. Source of HMW-GS did not affect compliance results; source of LMW-GS, however, did have an effect. The LMW-2 proteins strengthened dough to a greater extent than did LMW-1. Mechanisms involved in dough viscoelastic properties are described in terms of reversible physical crosslinks.

Introduction

Durum wheat (*Triticum durum*) is the commodity of choice for production of high quality pasta and for couscous. Strong gluten is considered an asset in durum wheat for production of pasta with good cooking quality (Feillet and Dexter 1996). Development of durum wheat lines with increased gluten strength for improved pasta texture has resulted in tenacious non-extensible gluten (Quaglia 1988). Durum wheat is increasingly gaining in popularity for production of flat breads and specialty breads, primarily in Mediterranean countries, stimulating interest in developing strong cultivars with a better balance between resistance to extension and extensibility for more general end-use applications (Liu et al. 1996).

Traditionally, physical properties of durum wheat dough have been determined using empirical dough mixing instruments such as the mixograph and farinograph, or by use of descriptive rheological methods such as the alveograph. While these instruments provide useful information to processors, they do not provide information on the fundamental viscoelastic nature of dough. Gluten composition is a major factor in determining durum semolina dough mixing strength and processing quality. Gluten has been characterized as having a bimodal distribution comprised of gliadin and disulfide linked glutenin polymers (Wrigley and Bekes 1999). Gliadin is often considered to contribute to dough extensibility and glutenin to dough elasticity. The relative proportions of gliadin and glutenin found in gluten affect dough characteristics, with higher relative proportions of glutenin corresponding to greater dough strength

(MacRitchie 1987). Glutenin itself is highly polydisperse, and may be reduced to release low molecular weight glutenin subunits (LMW-GS) and high molecular weight glutenin subunits (HMW-GS). When using a long fermentation baking procedure Gupta and co-workers (1992) found that 68 to 80% of the variation in dough extensibility, farinograph dough development time, and loaf volume could be accounted for by percentage of total glutenin in common wheat flour. In durum wheats varying in dough strength, Edwards et al. (2001) demonstrated that differences in fundamental viscoelastic properties corresponded to differences in total glutenin.

Although there are conflicting reports in the literature on associations between HMW-GS composition and durum gluten quality (Boggini and Pogna 1989, du Cros 1987, Ruiz and Carrillo 1995), correlative evidence suggests that LMW-GS composition does contribute to durum gluten quality. Studies demonstrated that γ-gliadin 42 and γ-gliadin 45 were useful predictors of poor and good pasta cooking quality (Damidaux et al. 1978, Kosmolak et al. 1980), and were later shown to be genetic markers for LMW-1 and LMW-2, respectively (Pogna et al. 1988, 1990). Several LMW subunit patterns are common to both LMW-1 and LMW-2. Of those that differ, LMW subunits 1+14, which are linked to γ-gliadin 45, had significant positive effects on gluten strength, while the presence of subunits 6+11 (linked to γ-gliadin 42) was negatively associated with gluten strength as measured by the SDS sedimentation method (Vásquez et al. 1996). Boggini and co-workers (1995) found that LMW-2 subunits were the major contributing factor to dough strength measured by the alveograph. A suggested explanation for the different quality attributes between the two LMW-GS types was that LMW-2 proteins

were expressed in greater amounts than LMW-1 types and that overall the effect on gluten quality was mainly a quantitative issue (Autran et al. 1987, D'Ovidio et al. 1999).

Abundant experimental evidence that wheat flour dough and gluten exhibit viscoelastic properties has been summarized in several review articles (Baird and Labropoulos 1982, Eliasson 1990, Faubion and Hoseney 1990). Edwards et al. (2001) recently described dough behaviour as somewhat analagous to a multimodal reversible gel with a broad range of molecular weight components involved crosslinks and/or entanglements. They found through creep testing that the entire elastic compliance curve shifted horizontally to lower values while steady state viscosity increased with increasing durum dough strength. Highly enhanced viscoelastic properties (e.g., high complex viscosities, low frequency dependence, stress relaxation that obeys power law dynamics as opposed to exponential decay) are characteristic of networks with reversible junctions, that are quite different from polymer melts with entanglements (Tanaka and Edwards 1992a). Of note is the characteristic of higher viscosity for lower molecular weight chains for physical gels with reversible crosslinks, which is the opposite of what would be expected in an entangled, uncrosslinked polymer melt. While both reversible gels and polymer melts are able to flow, the mechanism by which polymer melt flows is through disentanglement. Reversible gels are able to flow through rearrangement of the network via breaking and reformation of reversible crosslinks. The concept of breaking and reforming bonds during flow is somewhat similar to the theories proposed by Bloksma (1975) and Belton (1999) to explain the flow properties of dough. Bloksma (1975)

proposed that at a particular stress elastic deformation of gluten dough was related to the number of rheologically effective disulfide cross-links, and that viscous flow resulted from breaking and reformation of the disulfide bonds via a thiol-disulfide interchange reaction. Belton (1999) proposed the "loop and train" theory in which glutenin subunits, end-linked by disulfide bonds, form interchain associations via hydrogen bonding. Working or stretching of the dough extends the loops and the trains are pulled apart, allowing the proteins to slide along one another. Re-establishment of the train-loop equilibrium provides elastic recovery. Reversible gels are indistinguishable from a permanent network on time scales shorter than the average lifetime of a transient crosslink bond, but at longer times chains can break away and re-associate at another point, thus allowing for flow and for stress to relax (Leibler et al. 1991). Tanaka and Edwards (1992a,b,c) suggested in their model system of a transient network that hydrogen bonds, ionic associations, hydrophobic interactions, or other types of non-covalent interactions were possible participants in reversible crosslinks. Although disulfide bonds are covalent the lifetime of a bond relative to some practical time frame ultimately determines whether the bond is permanent or transient. It is well established that doughs flow in a measurable time, and that altering thiol-disulfide interchange reactions, for example by the addition of blocking agents such as NEMI (Ng et al. 1991, Schroeder and Hoseney 1978), affects dough flow properties. It therefore is reasonable to consider a thiol-disulfide linkage as a reversible crosslink in real time, and that dough may be considered somewhat analagous to a reversible gel. Glutenin polypeptide chains are assumed to potentially form one or more disulfide bonds at their –N and –C termini (Shewry et al. 1994). As a result, the number of

crosslinks would increase as glutenin average molecular weight decreases, because of the greater number of shorter chain lengths with disulfide bonds at each end, for a given protein content, suggesting that dough strength may be strongly influenced by intermediate molecular weight glutenin polypeptides.

The first objective of this work was to assess the contribution of gluten, and its gliadin, glutenin, LMW-GS and HMW-GS components to durum dough viscoelastic properties. The second objective was to ascertain whether strength variations amongst durum wheat varieties were related to qualitative differences between components or whether they resulted primarily from variations in quantity. We will attempt to compare dough linear viscoelastic properties with corresponding behaviours observed in synthetic polymers.

Materials and Methods

Wheats

Three cultivars of durum wheat with a wide range in strength were chosen for the study. Stewart 63 is an old Canadian variety no longer commercially grown possessing very weak gluten. AC Morse is a currently registered Canadian variety with moderately strong gluten properties, while Durex is an American desert durum with very strong gluten properties. All three were grown under identical environmental conditions at the Agriculture and Agri-Food Canada, Semiarid Prairie Research Centre, Swift Current, Saskatchewan and were kindly provided by Dr. J.M. Clarke. A composite sample of

commercially grown Canadian durum wheat was used to produce semolina as a base for enrichment studies.

Milling

All wheats were cleaned and tempered overnight to 16.5% moisture content. Wheats were milled following the procedure of Dexter et al. (1990) using a four-stand laboratory mill (Allis-Chalmers, Milwaukee, WI) in conjunction with a laboratory purifier (Black 1966) to produce semolina of approximately 66% extraction (on a clean wheat basis). The milling area was controlled for temperature (21°C) and relative humidity (60%).

Semolina Quality Tests

All analytical results are expressed on a 14% moisture basis. Moisture content was measured in a single-stage air oven using approved method 44-15a (AACC 2000). Protein content was determined by combustible nitrogen analysis (model FP-428, Leco Corp., St. Joseph, MI) calibrated against EDTA. Mixograph data were obtained on a 2g direct drive mixograph (National Manufacturing Division, TMCO, Lincoln, NE) at fixed water absorption of 50% (14% mb) and analyzed using Mixsmart software supplied with the instrument. Mixograph parameters were calculated as described by Khatkar et al. (1996). Relative proportions of gliadin and glutenin were measured by SE-HPLC (Nightingale et al. 1999). Gliadin composition was determined by acid-polyacrylamide electrophoresis (Whelan and Lukow 1990). Sodium dodecyl sulfate

polyacrylamide gel electrophoresis (SDS-PAGE) was used to characterize glutenins following the procedure of Lukow and co-workers (1994). Nomenclatures of Payne and Lawrence (1984) and Nieto-Taladriz et al. (1997) were used to classify HMW and LMW glutenin alleles, respectively. Dr. N. Ames (Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Canada) kindly performed the electrophoretic testing.

Gluten Extraction and Fractionation

Semolina was defatted with chloroform and air dried at room temperature. Gluten was extracted by initially mixing the defatted semolina with 1mM NaCl to form a gluten ball, then washing under a stream of distilled water to remove starch. The gluten ball was freeze-dried. Gliadin and glutenin were isolated from defatted semolina as described by Fu and co-workers (1996) and freeze-dried.

Prior to isolation of LMW-GS and HMW-GS from semolina, the monomeric proteins were removed (Fu and Kovacs 1999). The LMW-GS and HMW-GS were then extracted using the method described by Marchylo et al. (1989) with the following minor modifications: dithiothreitol concentration was lowered to 0.2% and HMW material was precipitated out by increasing 1-propanol concentration to 62.5% prior to centrifugation. The isolated protein fractions were then dialyzed against 0.1% acetic acid at 4°C for 72 hours and freeze-dried. Efficiency of the separation of HMW-GS from LMW-GS was monitored by RP-HPLC (Marchylo et al. 1989).

Enrichment Studies

All protein fractions were added to the base semolina (protein content 12.3% on 14% mb) at a level of 40 mg protein so that all comparisons were made on the basis of constant protein content. HMW-GS and LMW-GS were added only at 20 mg enrichment levels, because of lack of material. Mixograph testing was conducted on the control, and on all samples to which protein fractions had been added. Dough samples were prepared for rheological testing in the 2g mixograph as previously described (Edwards et al. 1999).

Frequency sweep and creep experiments were conducted using a Rheometrics SR500 controlled stress rheometer (Rheometric Scientific, Piscataway, NJ) in shear. The rheometer was fitted with 25mm serrated parallel plates, maintained at 25°C and gapped to 2.75mm. Excess dough was carefully trimmed and the exposed edge coated with silicon grease. A humidity chamber was also put in place to further prevent surface drying of the sample. Samples were rested for 15 minutes after loading prior to testing.

Oscillatory measurements were conducted at 0.35% strain, within the linear viscoelastic regime, over a frequency range of 0.1 to 10 Hz. Creep tests were conducted within the linear viscoelastic regime at 20 Pa applied stress. Each dough sample was subjected to creep for 10,000s, sufficient time to reach steady state flow (Edwards et al. 2001). The time required for testing combined with the number of replicates made recovery experiments impractical. All creep results were obtained in the linear regime, therefore

results are presented as creep compliance, J(t), and were modeled using a six element Burgers model (see Chapter 4).

Experimental Design

Protein extraction and fractionation were performed in duplicate. The base semolina and base semolina plus gluten and its components were tested in duplicate by mixograph. Oscillatory and creep tests were conducted at least in duplicate for each fraction and reported as averages. Coefficients of variation between replicate compliance tests were on average 10%, and between replicate oscillatory tests were 4%.

Results and Discussion

The three durum cultivars used in the study ranged in dough mixing strength from very weak to very strong, as demonstrated by their mixograms (Fig. 5.1), and this may be related at least in part to differences in relative proportions of gliadin and glutenin among donor varieties (Table 5.1). As the relative proportion of glutenin decreased there was an accompanying decrease in dough mixing strength.

Stewart 63 differed in its γ-gliadin, HMW-GS and LMW-GS composition from both Durex and AC Morse (Table 5.1). Durex and AC Morse both contained γ-gliadin 45, a LMW-GS marker consistent with LMW-2 patterns which are associated with good pasta making quality. Durex and AC Morse were identical in their HMW-GS and

LMW-GS patterns. Stewart 63 contained γ-gliadin 42, a marker for the poor quality LMW-1 glutenin type (Pogna et al. 1988, 1990).

Table 5.1 Analytical Results for Durum Semolina

	Stewart 63	*AC Morse*	*Durex*
Protein[a], %	12.1	13.4	12.4
γ-Gliadin Type	42	45	45
SDS-PAGE			
HMW-GS	7+8	6+8	6+8
LMW-GS			
Glu-A3	5	6,10	6,10
Glu-B3	8,9,13,16	2,4,15,19	2,4,15,19
Glu-B2	12	12	12
SE-HPLC			
Gliadin, %	62.5	55.5	49.2
Glutenin, %	37.5	44.5	50.8

[a] Corrected to 14% mb.

Gluten Enrichment

Gluten extracted from each of AC Morse and Durex, and added on a constant protein basis (40 mg), resulted in an overall increase in mixing strength compared to the base semolina as measured by mixograph (Fig. 5.1). Addition of gluten from Stewart 63 to the base semolina had little effect on mixing strength (Fig. 5.1). Overall, mixing strength of the gluten enriched doughs ranked according to the strength of the donor variety.

When dynamic measurements were conducted on the same doughs rankings were similar to mixograph results (Fig. 5.2). Dough enriched with Stewart 63 gluten had

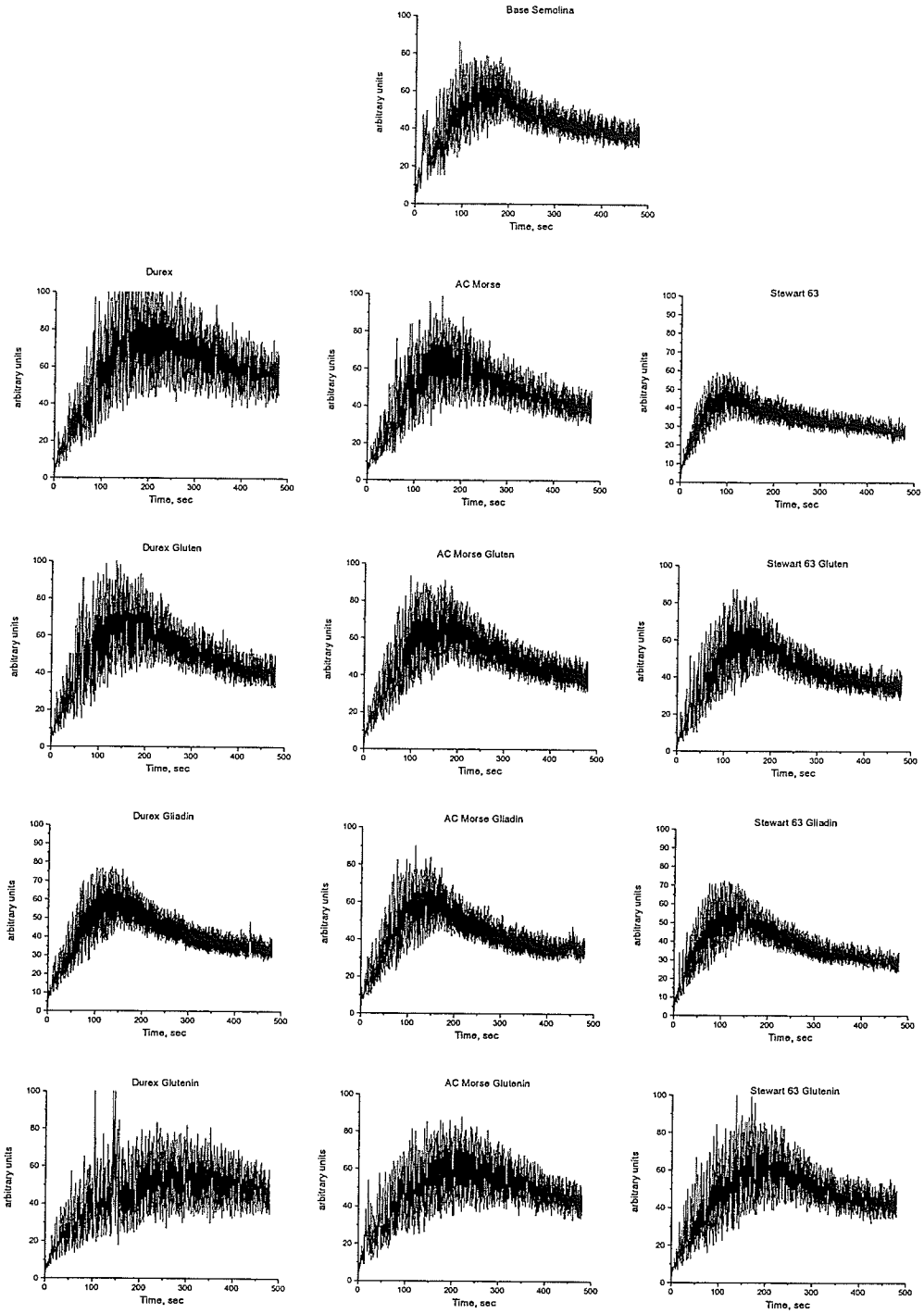

Figure 5.1. Mixograph curves of the base semolina, donor varieties, and base semolina enriched with 40 mg (constant protein basis) gluten, gliadin or glutenin from each of the donor varieties.

lower elastic modulus (G') than the base semolina on its own (Fig. 5.2a). Addition of gluten from AC Morse had little effect on base semolina G'. Enrichment with Durex gluten led to a slight increase in G' over that of the base semolina. Addition of gluten from all three varieties reduced the viscous modulus (G") in comparison with the base semolina, Stewart 63 to the greatest extent and Durex the least. In comparing the relative contributions of the elastic and viscous moduli (tan δ = G"/G'), the two stronger gluten varieties had lower tan δ than the base semolina, indicative of greater contribution of the elastic component (Fig. 5.2b). Stewart 63 gluten addition, on the other hand, imparted a slightly greater contribution of the viscous component in the middle frequency range, having a slightly higher tan δ than the base semolina. In other words, addition of Stewart 63 gluten had a slight weakening effect on the dough, which may be related to the greater relative proportion of gliadin found in Stewart 63 in comparison with the other two varieties (Table 5.1).

Creep compliance testing of the gluten enriched doughs demonstrated that compliance was reduced in all cases relative to the base semolina (Fig. 5.3a), with the three donor varieties ranking in order of strength corresponding with mixograph curves. Dough enriched with gluten from the strongest variety, Durex, exhibited the greatest resistance to deformation with lowest overall compliance (Fig. 5.3a) and highest η_0 (Table 5.2). Enrichment with Stewart 63 gluten resulted in a downward shift of J(t), even though under dynamic testing conditions its addition appeared to slightly weaken dough. Addition of 40 mg gluten to dough increased dough protein content by approximately 2%. Increasing the relative amount of protein in the dough provided a greater source of

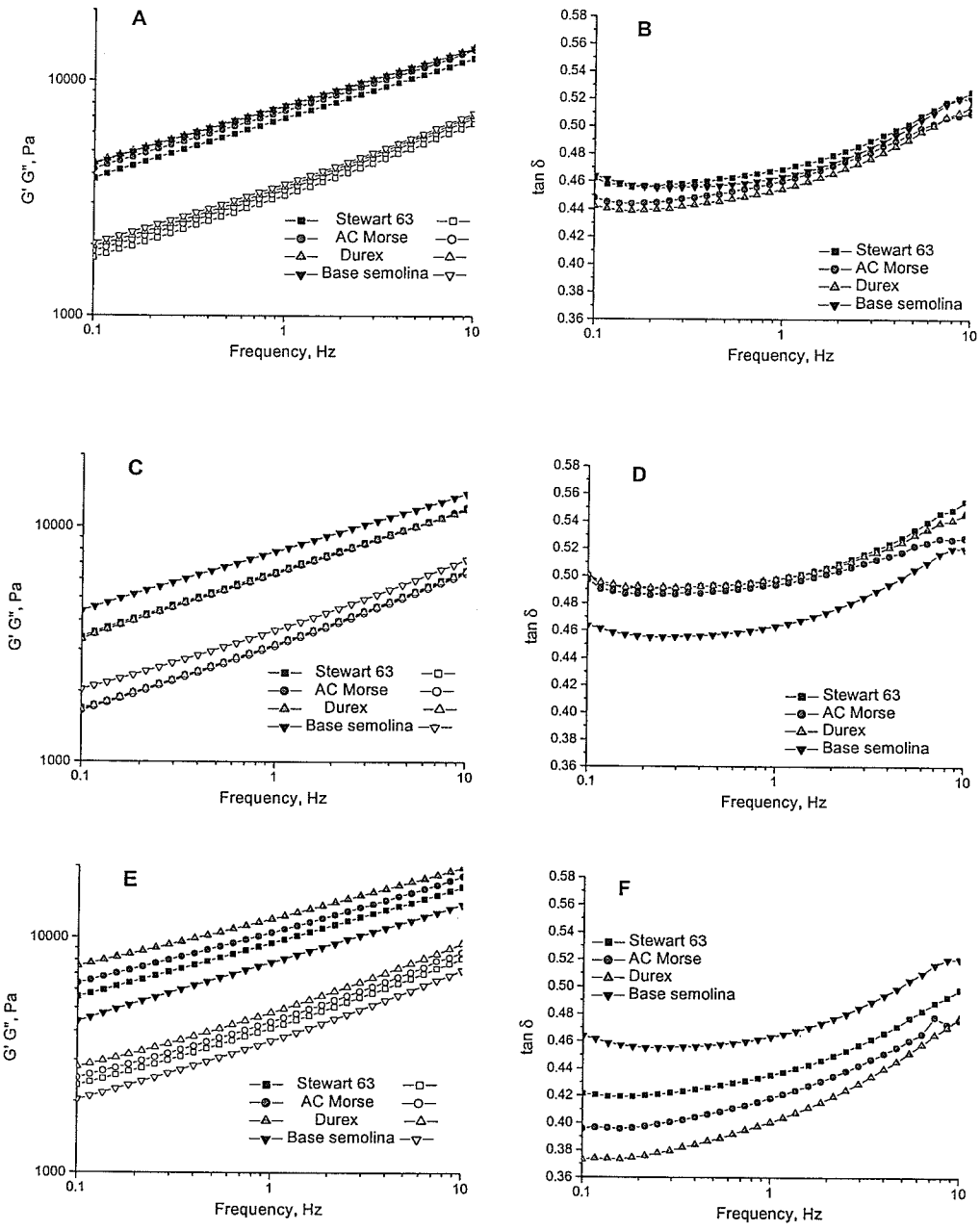

Figure 5.2. Dynamic moduli (closed symbols = G', open symbols = G") and tan δ for the base semolina dough and doughs enriched with 40 mg (constant protein basis) gluten (A & B), gliadin (C & D) or glutenin (E & F) from each of the three donor varieties.

material for production of a three-dimensional protein network, and therefore, a greater density of crosslinks per unit volume of dough. Edwards and co-workers (2001) found

that durum dough compliance curves shifted horizontally to lower values accompanied by increasing steady state flow viscosity (η_0) with increasing dough strength, which they related to a greater density of physical crosslinks and/or entanglements.

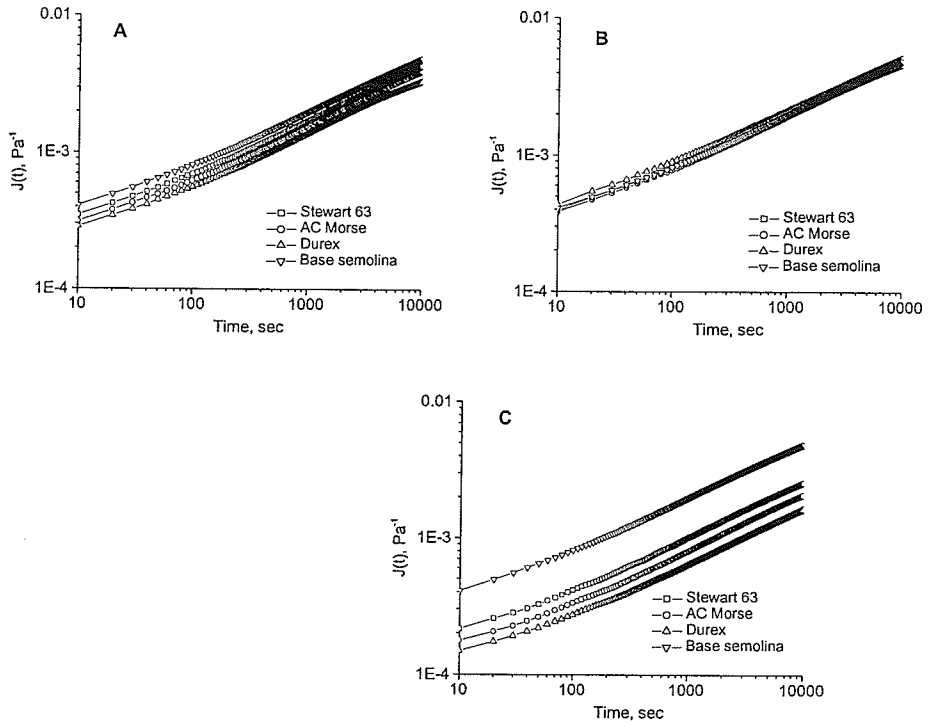

Figure 5.3. Creep compliance curves for the base semolina dough and doughs enriched with 40 mg (constant protein basis) (A) gluten, (B) gliadin and (C) glutenin from each of the three donor varieties.

Instantaneous compliance (J_1) has been attributed to the instantaneous bending and stretching of bonds upon application of stress, plus any secondary viscoelastic response arising from the orientation of very localized polymer chain segments or side groups that initially contribute to the deformation (Plazek and Frund 2000). Ratios of instantaneous compliance obtained for the enriched doughs relative to the base semolina, where the base semolina J_1 (4.89×10^{-4} Pa^{-1}) was taken to be 100%, were 67% for Durex, 76% for AC Morse and 84% for Stewart 63. It would be reasonable to

interpret the reductions in J_1 with increasing dough strength as indicative of an increase in the number of initial elastically effective network chains terminated primarily by disulfide crosslinks with the understanding that any entrapped entanglements could also contribute to short time elasticity. Gluten from all three varieties contributed additional crosslinks or entanglements to the dough.

Table 5.2 Instantaneous compliance (J_1) and steady state viscosity (η_0) model parameters for doughs enriched with protein fractions

	Fraction[a]	*Durex*	*AC Morse*	*Stewart 63*
J^1, Pa^{-1}	Gluten	3.32×10^{-4}	3.73×10^{-4}	4.15×10^{-4}
η_0, Pa.s	Gluten	7.79×10^{6}	6.62×10^{6}	5.95×10^{6}
J^1, Pa^{-1}	Glutenin	1.72×10^{-4}	2.06×10^{-4}	2.50×10^{-4}
η_0, Pa.s	Glutenin	15.8×10^{6}	14.3×10^{6}	10.6×10^{6}
J^1, Pa^{-1}	Gliadin	5.29×10^{-4}	4.61×10^{-4}	4.79×10^{-4}
η_0, Pa.s	Gliadin	5.75×10^{6}	5.78×10^{6}	5.26×10^{6}
J^1, Pa^{-1}	HMW-GS	3.41×10^{-4}	3.79×10^{-4}	3.57×10^{-4}
η_0, Pa.s	HMW-GS	8.00×10^{6}	7.57×10^{6}	9.90×10^{6}
J^1, Pa^{-1}	LMW-GS	2.71×10^{-4}	2.54×10^{-4}	3.84×10^{-4}
η_0, Pa.s	LMW-GS	7.27×10^{6}	7.66×10^{6}	7.24×10^{6}

[a] Gluten, gliadin and glutenin added at 40mg protein, HMW-GS and LMW-GS added at 20 mg protein

One of the advantages of creep testing is that elastic character and viscous flow may be separated and their influence considered individually. Total elastic or steady-state recoverable compliance (Fig. 5.4), comprises both the instantaneous and retarded elastic components and indicates strain resulting from polymer chain orientation (Plazek and Frund 2000). Elastic compliance was calculated by subtracting the steady state flow viscosity term, t/η_0, from J(t). By further subtracting the instantaneous compliance, J_1, the retarded elastic component was isolated (Fig. 5.4). Removal of the steady state flow viscosity term reduced the differences between donor varieties, particularly at longer

times, although all three curves remained lower than the base semolina to which they had been added and continued to rank in order of strength of the gluten donor variety.

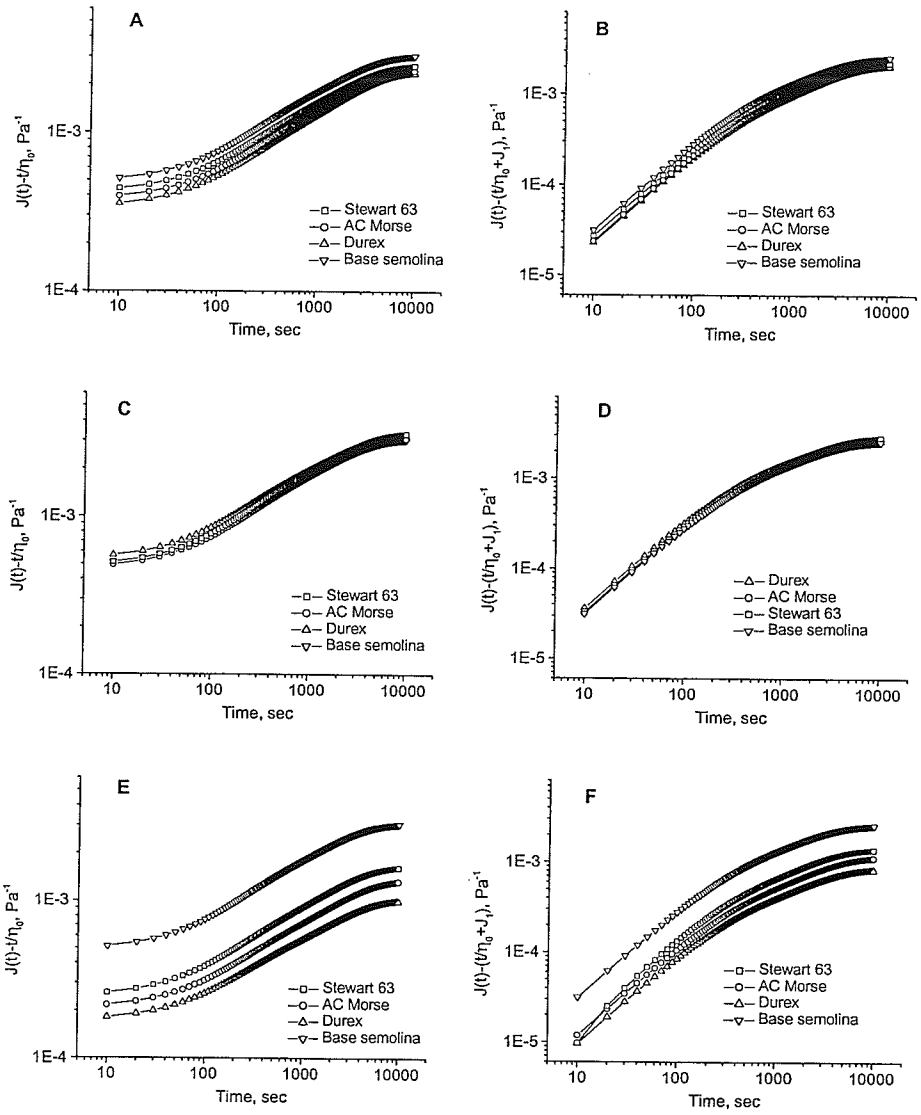

Figure 5.4. Elastic compliance ($J(t)-t/\eta_0$) curves (A, C, E) and retarded elastic compliance ($J(t)-(t/\eta_0+J_1)$) curves (B, D, F) for the base semolina dough and doughs enriched with 40 mg (constant protein basis) gluten (A & B) , gliadin (C & D) and glutenin (E & F) from each of the three donor varieties

Differences among donor varieties were to some extent affected by factors contributing to viscous flow, which may be related to the relative proportion of gliadin in the gluten fraction of each donor. Upon removal of J_1, differences among donors were essentially eliminated, and the spread between them and the base semolina curve were greatly reduced. The period of retarded elasticity, intervening instantaneous compliance at initial onset of stress and the period where the sample has attained steady state flow, differed very little among donor varieties, suggesting that density of initial elastically effective crosslinks was a primary factor affecting gluten strength. Tanaka and Edwards (1992a,b,c) in their work on transient network theory demonstrated with model systems that networks containing reversible crosslinks, intervened by chains that were too short to participate in entanglements, exhibited highly enhanced viscosity and elasticity.

Gliadin and Glutenin Enrichment

Mixograph dough strength indicators (mixing time, bandwidth at peak, work input) showed a slight weakening of the curve with addition of gliadin relative to the base semolina (Fig. 5.1). Gliadin addition reduced dynamic moduli in all cases, with an accompanying increase in tan δ of approximately 10%, but essentially no differentiation between donor varieties was observed (Fig. 5.2c, d). The lack of an effect on creep compliance, regardless of donor variety (Fig. 5.3b), demonstrated the inability of gliadin to form a network structure, while dynamic measurements highlighted the contribution of gliadin to the viscous nature of dough (Scanlon et al. 1990). There was

no difference in elastic compliance among donor varieties or between them and the base semolina (Fig. 5.4). Gliadin has previously been found to increase dough extensibility under high stress, large deformation conditions (Uthayakumaran et al. 2000a) and to weaken dough mixing strength (Skerritt et al. 1996). We had expected to find an increase in J(t) with addition of gliadin, given its behaviour under dynamic testing conditions and the established effects under large deformation testing. It is probable that in its native state gliadin acts as a compatibilizing agent that enhances gluten viscous flow or extensibility. In the native state gliadin and glutenin may have a required conformational arrangement, but with their addition to dough the required conformation may not be fully realized, reflected by the lack of change in creep compliance of dough enriched with gliadin.

Glutenin, in contrast to gliadin, had a dramatic strengthening effect on mixograph curves, and stiffened the dough leading to increased dynamic moduli, reduced tan δ and lower creep compliance. Addition of glutenin increased overall mixing strength with greater time to peak, increased bandwidth at peak, and increased work input (Fig. 5.1). Work input ranked according to the dough strength of the donor variety, as did dynamic moduli and tan δ (Fig. 5.2). Tan δ was reduced by as much as 20% with addition of glutenin, confirming its very strong contribution to dough elasticity.

Creep compliance of glutenin enriched doughs was reduced relative to the base semolina dough (Fig. 5.3), and ranked in order of strength of the donor varieties, but the differences were substantially magnified compared to the effect obtained from gluten

enrichment. The reduction in J_1 and increase in η_0 were quite striking (Table 5.2). Addition of Durex glutenin resulted in J_1 that was only 35% that of the base semolina, with a 3-fold increase in η_0 (Table 5.2). AC Morse and Stewart 63 glutenin reduced compliance to a lesser extent, with J_1 of 42% and 51% that of the base semolina and η_0 increased 2.7 and 2.0 times, respectively. Assuming that glutenin behaves as a reversible gel (Edwards et al. 2001, Mulvaney et al. 2001), it is reasonable to consider that concentration and rate of dynamic interchange of reversible crosslinks influenced viscous flow. It is therefore also reasonable to assume that Durex glutenin had a greater concentration of reversible crosslinks than AC Morse, and that Stewart 63 glutenin had the lowest concentration.

Removal of the viscous flow term (t/η_0), to obtain the elastic compliance curves did not alter the ranking of donor varieties for either gluten or glutenin enriched dough (Fig. 5.4a, e). Analysis of glutenin retarded elastic compliance curves (Fig 5.4f) showed that at short times differences among donor varieties were eliminated by subtraction of J_1. However, over the time scale of the test, differences reappeared, ranking according to donor strength, again suggesting differences in density of reversible crosslinks among donor varieties.

It was interesting to find that differences in dynamic moduli and compliance remained between Durex and AC Morse when doughs were enriched with a constant amount of glutenin protein, as they share the same electrophoretic pattern of glutenin subunits (Table 5.1). The differences in strength between the two varieties were, therefore, not

related solely to differences in gliadin-to-glutenin ratio. Logically, the primary source of variation between varieties would be from differences in molecular weight distribution within the polydisperse glutenin polymer. Even though Durex and AC Morse contain the same glutenin subunits, differing proportions of these subunits would lead to different average molecular weights, affecting concentration of crosslinks.

HMW and LMW Glutenin Subunits

HMW-GS and LMW-GS were isolated and added to the base semolina to identify the contribution of each to dough mixing strength and to LVP. Reversed-phase HPLC of the extracts indicated that the separation techniques resulted in almost complete removal of ω-gliadins and very clean separation of the LMW and HMW glutenin components (Fig. 5.5). Addition of HMW-GS to dough increased mixograph strength relative to the base semolina on its own (Table 5.3). In all cases, peak dough resistance, bandwidth at peak dough resistance, and mixing stability increased. Work input to peak resistance remained the same as the base semolina or increased. Veraverbeke et al. (1998) found that addition of common wheat HMW-GS to a base flour had a positive effect on dough strength. There was some improvement in dough mixing strength parameters with the addition of LMW-GS. Peak height and work input were reduced, but were offset by reductions in bandwidth breakdown and increased mixing stability (Table 5.3). Stewart 63 LMW-GS imparted less of an improvement in dough strength than did Durex and AC Morse LMW-GS. Sissons et al. (1998) found that incorporation

of LMW-GS from a common wheat variety had a positive effect on mixograph strength parameters, while Fu (1996) found that LMW-GS incorporation had marginal effects.

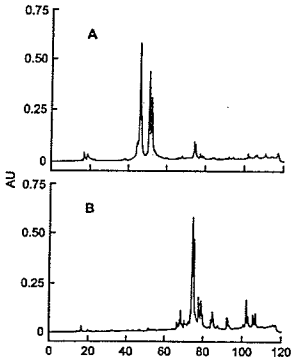

Figure 5.5. Reversed-phase high performance liquid chromatography profiles of typical (A) HMW-GS and (B) LMW-GS isolates from durum wheat.

Regardless of the source of HMW-GS, compliance curves were similar (Fig. 5.6a). All exhibited reduced compliance compared to the base semolina, indicating a contribution to overall strength of the dough through formation of a network structure. As mentioned earlier, there is not agreement in the literature about the contribution of HMW-GS to durum gluten strength. The data from this study indicate that HMW-GS did contribute positively to dough strength, but that there were not differences between varieties containing subunits 7+8 and 6+8 in terms of network formation when compared on a constant protein basis.

Table 5.3. Mixograph properties of durum dough enriched with 20 mg HMW-GS or LMW-GS[1]

Parameter[2]	Base Semolina	Durex		AC Morse		Stewart 63	
		HMW-GS	LMW-GS	HMW-GS	LMW-GS	HMW-GS	LMW-GS
MT (min)	2.86	3.28 ± 0.67	2.68 ± 0.02	2.96 ± 0.37	2.82 ± 0.45	3.55 ± 0.88	3.09 ± 0.20
PDR (AU)	58.7	55.4 ± 1.8	47.9 ± 0.8	57.8 ± 1.1	48.1 ± 1.3	56.6 ± 0.5	49.9 ± 1.4
BWPR (AU)	26.2	31.6 ± 3.5	27.6 ± 1.7	30.9 ± 1.2	27.2 ± 1.0	29.7 ± 3.4	26.6 ± 1.6
BWBD (AU)	11.4	7.8 ± 5.4	8.7 ± 3.7	12.1 ± 3.5	4.1 ± 0.5	9.8 ± 1.1	10.5 ± 1.6
MS (AU)	14.8	23.8 ± 1.9	19.0 ± 2.0	18.8 ± 2.3	22.4 ± 2.1	19.9 ± 2.3	16.2 ± 3.2
WI (AU)	120	131 ± 29	90 ± 3	120 ± 18	97 ± 27	147 ± 48	108 ± 1

1. Results are averages of duplicate measurements ± standard deviation.
2. MT = mixing time; PDR = peak dough resistance; BWPR = band width at peak resistance; BWBD = bandwidth breakdown; MS = mixing stability; WI = work input to peak resistance

Linear viscoelastic response of doughs enriched with LMW-GS at equivalent protein content was dependent on the protein donor (Fig. 5.6). AC Morse and Durex both contain LMW-2 type subunits that are associated with good gluten quality, while Stewart 63 has LMW-1 type associated with poor gluten quality. Analyses in several

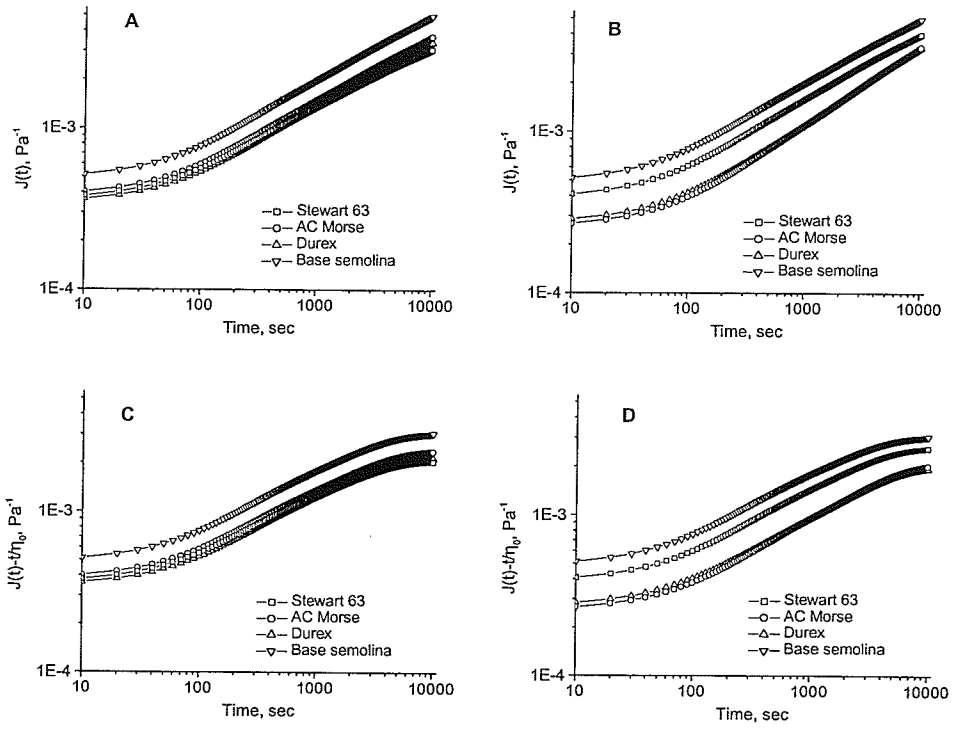

Figure 5.6. Creep compliance curves for the base semolina dough and when enriched with 20 mg (constant protein basis) (A) HMW-GS and (B) LMW-GS; and elastic compliance curves for the base semolina dough and dough enriched with (C) HMW-GS and (D) LMW-GS isolated from each of the three donor varieties.

studies have demonstrated that LMW-2 is expressed in greater amounts than LMW-1, and it was suggested that differences in relative quantities could explain gluten strength differences (Autran et al. 1987, D'Ovidio et al. 1999, Masci et al. 1995). In this study,

LMW-GS was added on a constant protein basis and the differences remained between the two LMW types indicating that qualitative differences also played a role.

D'Ovidio and co-workers (1999) sequenced two allelic genes located on *Glu-B3* encoding for LMW-2 and LMW-1 proteins and found that they differed by the insertion of 13 amino acids in the repetitive domain of LMW-2. The repetitive domain showed 92.9% homology between LMW-1 and LMW-2 patterns, with the LMW-2 protein having a calculated molecular weight of 42,242. The presence of a molecular weight 42,000 band (the largest protein) in LMW-2 SDS-PAGE pattern, encoded by chromosome 1B at the *Glu-B3* locus, and missing in LMW-1 patterns was the main difference identified between the two LMW types (Ruiz and Carrillo 1995). The 13 amino acid segment identified by D'Ovidio and co-workers (1999) that was present in LMW-2 but missing from LMW-1 was rich in glutamine, which would provide additional H bonding sites available to participate in transient crosslinks. It appears probable from our data that molecular structural differences imparted varying densities of crosslinks that contributed to qualitative differences between LMW-1 and LMW-2 types.

In comparisons amongst varieties, the compliance of LMW enriched dough from both LMW-2 lines (AC Morse and Durex) was similar, while compliance of their total glutenin differed. Since the response of doughs enriched with constant amounts of LMW-GS isolated from Durex and AC Morse was similar, the inference could be made that the average molecular weight within the LMW-GS isolates was similar, with

roughly equivalent concentration of reversible crosslinks per unit volume. The same could be suggested for HMW-GS isolated from all three varieties. The response of total glutenin isolated from the LMW-2 types differed suggesting that average molecular weight of the total glutenin of the two varieties differed, probably as a result of different relative proportions of LMW-GS and HMW-GS.

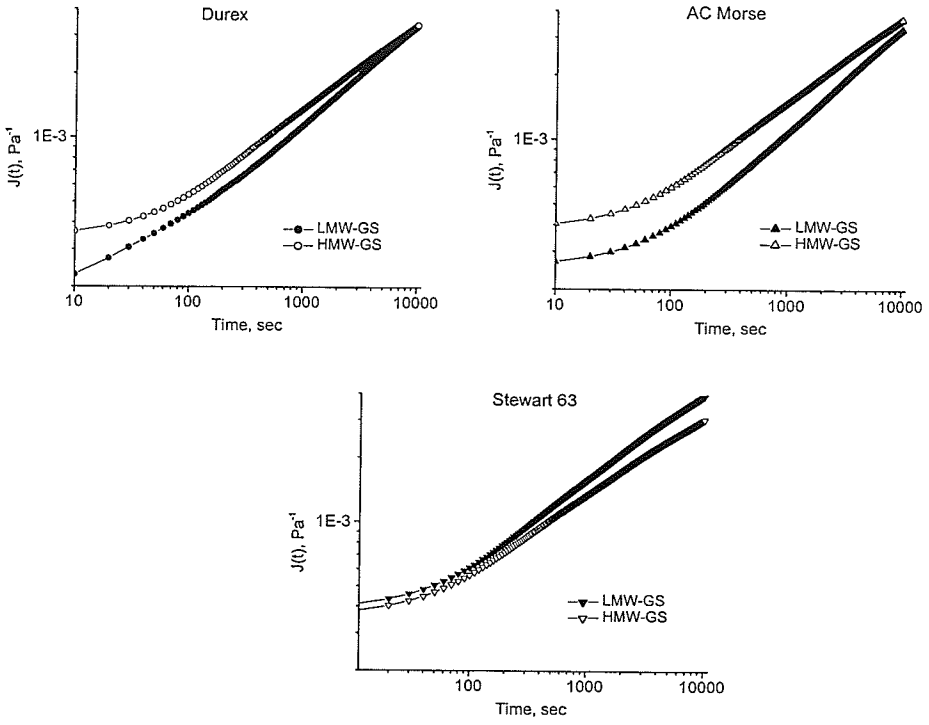

Figure 5.7. Comparison of creep compliance of dough enriched with isolated HMW-GS and LMW-GS from Durex, AC Morse and Stewart 63 on a varietal basis.

The LMW-GS isolated from both Durex and AC Morse had lower J(t) curves than their respective HMW-GS when compared at equivalent protein content (Fig. 5.7), indicating a greater contribution of LMW-GS to dough strength for these two varieties. Stewart 63 curves were the opposite, with the HMW-GS curves slightly lower than the LMW-GS

curve (Fig. 5.7). The contribution of LMW-GS becomes even more relevant when considering that in its native state, durum glutenin is composed of approximately 80% LMW-GS and 20% HMW-GS (Payne et al. 1984).

Comparison with Model Polymers

A study published by Plazek and Frund (2000) on recoverable creep compliance of associative polymers and linear polymer solutions provides an interesting comparison for the linear viscoelastic responses of dough enriched with gluten, glutenin and its subunits. In their study, Plazek and Frund (2000) looked at, among other things, the effects of molecular weight and concentration on both unassociated polymer and the polymer with associating groups. The base polymer used in their study was polyoxyethylene (POE) having a molecular weight of approximately 8000 g/mole with –OH at each end. Unassociated high molecular weight materials (up to about 2×10^6 g/mole) used in the study were constructed by end-linking polymerization of POE to produce long linear molecules. The associated polymers consisted of linear POE backbones terminated with hexadecyl hydrophobic end groups rather than -OH groups. The authors found that at a constant polymer concentration and temperature, increasing molecular weight of the unassociated polymer led to reduced recoverable or elastic compliance and increased steady state flow viscosity. As concentration was increased, at a given molecular weight, compliance was reduced and time to reach steady state was shifted to longer times. In comparison, associative polymers were characterized by a decrease in steady state flow viscosity, and an increase in recoverable creep compliance

with increasing molecular weight at constant concentration and temperature, which was attributed to a decrease in the number of associating groups for the same concentration. As the concentration of associative polymer was increased, at a given molecular weight, compliance decreased, although the time to reach steady state compliance remained unchanged due to loss of long-time mechanisms. Mulvaney and co-workers (2002) put forward the suggestion that wheat flour and semolina doughs subjected to a range of applied shear stresses share some similarities with associative polymers, as opposed to entanglement networks. Our data appear to lend further credence to that hypothesis.

Creep compliance curves of dough enriched with a constant quantity of gluten protein from three durum varieties of varying strength resulted in decreasing elastic compliance with increasing dough strength of the donor variety (Fig 5.4a). Essentially, as dough strength increased there was an increase in the concentration of glutenin polymer (Table 5.2) and an accompanying decrease in compliance, similar to the description of Plazek and Frund (2000) for both associative and unassociated polymers. There was not a change in the time required to reach steady state as glutenin concentration increased, a characteristic behaviour of an associative polymer. Adding glutenin directly to dough also resulted in reduced compliance that was concentration dependent with little change in time required to reach steady state (data not shown). All dough elastic compliance curves had a somewhat sigmoidal shape (Figs. 5.4, 5.6, 5.7), a pattern noted by Plazek and Frund (2000) for associative polymers. All dough elastic compliance curves also exhibited a single transition from a short time network plateau (<100 s) that possibly results from some type of associative network, to a second higher plateau at steady state

compliance (after approximately 3000 s). Viscous compliance was essentially negligible at short times for all dough samples, independent of protein isolate enrichment. When viscous compliance and elastic compliance were plotted independently, the viscous compliance curve intersected the elastic compliance curves in the second plateau region, indicating the overall high viscosities for all doughs at longer times (Fig. 5.8).

Perhaps the most interesting comparisons are to be made with HMW-GS and LMW-GS enriched doughs, where there were known differences in molecular weight. Low molecular weight glutenin subunits isolated from the two stronger varieties, Durex and AC Morse, exhibited lower elastic compliance than their respective HMW-GS when added on an equivalent protein basis to the base semolina (Fig. 5.7), behaviour similar to that seen with associative polymers of differing molecular weights. Dough enriched with LMW-GS from Stewart 63 was similar in compliance to that enriched with its HMW-GS over short time, after which LMW-GS compliance increased at a greater rate. As described earlier, this wheat is a LMW-1 type that typically has poorer dough strength characteristics than the LMW-2 types. We have attributed the weaker strength to a lower density of reversible crosslinks, comprised mainly of disulfide bonds and hydrogen bonds. Lower crosslink density per unit volume would be analagous to increased molecular weight of an associative polymer with decreased number of associative groups for a given concentration. It appears that viscosities of HMW-GS and LMW-GS may be additive in nature, since the viscosity of each on its own (at 20 mg enrichment) is approximately half that of dough enriched with 40 mg total glutenin (Table 5.2).

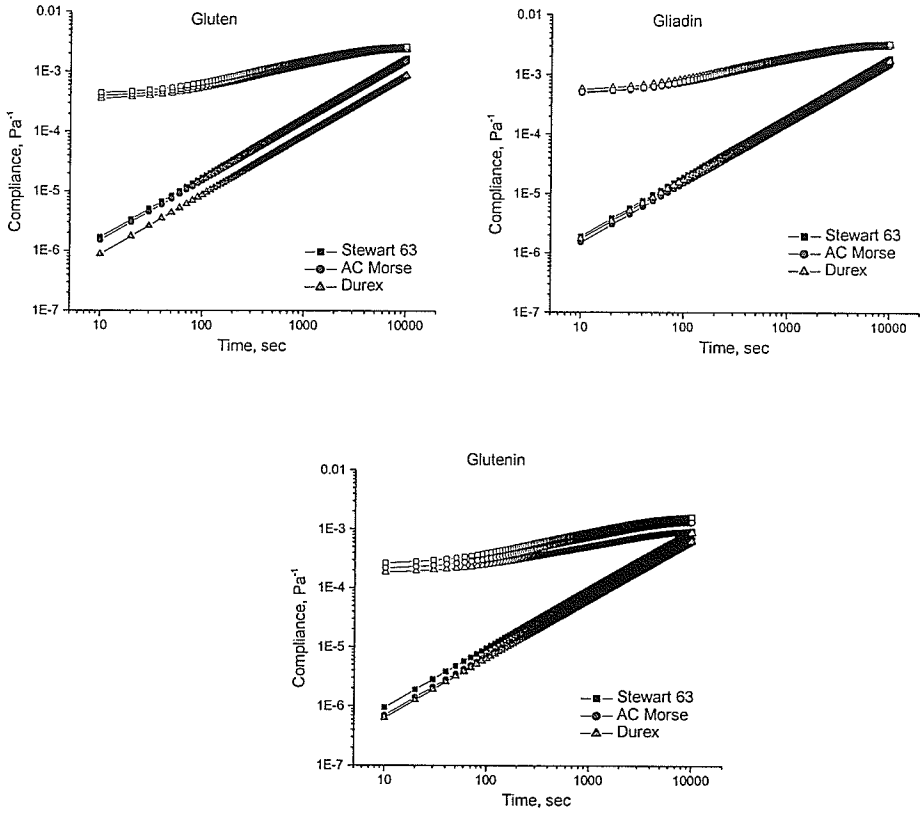

Figure 5.8. Elastic compliance, $J(t)-t/\eta_0$, (open symbols) and viscous compliance, t/η_0, (closed symbols) plotted together for comparison for gluten, gliadin and glutenin isolated from the three donor varieties.

Conclusions

Gluten contributed to durum dough viscoelastic properties to varying degrees dependent on both gliadin-to-glutenin ratio and LMW-GS type of the source cultivar. Greater proportions of glutenin in the source gluten increased dough strength. Gliadin contributed to dough viscous flow properties as shown by reduced dynamic moduli and increased tan δ when added to the base semolina. Gliadin was unable to form any network structure, having essentially no effect on J(t), and weakened dough mixing strength as measured by mixograph. Source of the gliadin made no difference in dough linear viscoelastic response. Glutenin was the primary contributor to dough strength and

elasticity, but when added on constant protein basis differences among donor varieties persisted, confirming that quantitative variation found in gluten was not the sole basis for differences in LVP among varieties. In all cases, addition of glutenin to the base semolina increased dough strength and elastic character, with increased dynamic moduli, lower tan δ and reduced J(t) with absolute values of the measurements dependent on the source variety. Upon further fractionation of glutenin into its HMW-GS and LMW-GS components we were able to isolate the effects of both on dough strength. Enrichment with either fraction on a constant protein basis increased overall dough strength. Source of HMW-GS did not affect J(t), but it did have minor effects on mixograph parameters. Source of LMW-GS, on the other hand, did influence J(t). LMW-GS from the two varieties that were of the LMW-2 type, Durex and AC Morse, exhibited much lower compliance than Stewart 63, the LMW-1 type. At constant protein there were not differences in J(t) between LMW-GS from Durex and AC Morse, inferring similarities in structure leading to similar densities of reversible crosslinks. There were differences in J(t) with addition of their respective total glutenin fractions, suggesting that differences in molecular weight distribution within the glutenin polymer provided Durex glutenin with a greater density of reversible crosslinks for the same concentration of protein compared with AC Morse. Compliance of LMW-GS was lower than the respective HMW-GS for both the LMW-2 types, confirming LMW-GS to be the major contributor to durum dough strength.

Decreasing J(t) was observed with increasing concentration of durum glutenin, and with decreasing molecular weight when comparing HMW-GS and LMW-GS for the LMW-2

types, a behaviour that corresponds with that of associative polymers based on the work of Plazek and Frund (2000). All samples enriched with gluten, glutenin and its components exhibited high viscosity, also typical of reversible gels (Tanaka and Edwards 1992a,b,c) and associative networks (Plazek and Frund 2000), in which the number of crosslinks per unit volume define network strength and extensibility.

CHAPTER 6

Starch Participation in Durum Wheat Dough Viscoelastic Properties

Abstract

The contribution of starch to dough rheological properties has been largely overshadowed by the role of gluten, and thus has received much less attention in comparison. The influence of starch granule surface properties on durum wheat dough linear viscoelasticity was investigated, and surface interactions between starch granules and gluten quantified, using a model system. Proportions of starch were substituted in dough on a volume basis with an inert filler, glass powder, having a similar particle size range to starch, and the doughs were subjected to dynamic and creep measurements. Dough linear viscoelastic properties were weakened upon substitution of starch with glass powder up to 50% substitution, inferring a contribution arising from adhesion of the starch granules to gluten protein at their interface, and confirming the inert nature of glass powder. Surface modification of glass powder by adsorption of BSA ameliorated the rheological response of the gluten-glass powder dough. Heat treatment of starch enhanced elastic character of gluten-starch dough, probably through promotion of hydrophobic interactions with hydrated gluten protein. Masking hydrophobic sites on the surface of heat-treated starch granules by adsorption of BSA reduced starch-gluten interface interactions, confirming the importance of starch granule surface properties in dough rheological behaviour.

Introduction

Wheat flour dough can be considered a composite material in which gluten forms the continuous matrix and starch granules act as filler particles within that matrix. Assuming insignificant contribution by minor components, the properties of the composite will depend on the material properties of the protein matrix and starch filler and degree of adhesion and interaction between the starch granules and protein matrix. Material properties that would contribute to rheological properties include the total volume occupied by starch granules, their size distribution and any interaction between them. Although starch constitutes the largest volume fraction of solids in dough, comprising approximately 75% (Colonna et al. 1990), limited work has been published describing the contribution of starch to dough rheological properties. In particular, there is little information on starch-protein surface interactions, although the importance of starch granule surface properties to wheat flour dough rheological properties has been established (Larsson and Eliasson 1997).

Starch-protein surface interactions have been discussed in relation to wheat hardness and their effect on milling quality (Greenblatt et al. 1995, Greenwell and Schofield 1986). The presence of the protein friabilin on the starch granule surface has been associated with softer endosperm texture (Darlington et al. 2000). Hard wheats tend to break around endosperm cell walls and through starch granules during milling or grinding whereas breakage occurs through cell contents and around starch granules in wheats with soft endosperm texture (Simmonds 1974). Barlow et al. (1973) hypothesized that the strength

of adhesion between starch granules and protein was responsible for differences in endosperm hardness after finding little difference in indentation hardness between starch and protein components of hard and soft wheats.

Rasper and deMan (1980) studied the effect of starch granule size on dough tensile properties using starch from different botanical sources and glass powder. They concluded that particle size did have an effect, but that water-binding capacity of different starches may exert a greater effect. Larsson and Eliasson (1997) studied the influence of starch granule surface properties on rheological behaviour of wheat flour doughs by diluting a flour with treated and untreated starch to a fixed protein content. They found that heat-treatment and surface modification of the starch by protein or lecithin adsorption altered dough rheological properties, and concluded that starch granule surface properties are important for dough rheological behaviour.

Our objective was to investigate the contribution of starch granule surface interactions with gluten protein on the linear viscoelastic properties of durum wheat dough. The role of starch in durum wheat dough linear viscoelastic properties was investigated by incrementally substituting starch with an inert filler, glass powder, with a particle size range similar to starch, and subjecting the dough samples to dynamic and creep measurements. In addition, to investigate the role of starch in gluten-starch interactions we modified the surface of the glass beads and starch granules by adsorption of a protein solution and by heat treatment.

Materials and Methods

Wheat

Three cultivars of durum wheat grown at Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Centre, Swift Current, Canada were kindly supplied by Dr. J.M. Clarke and were used as the source of starch throughout this study. The three included Durex, an American desert durum, AC Morse, a registered Canadian variety, and Stewart 63, an old Canadian variety that is no longer commercially produced. A sample of commercially grown Canadian durum wheat was used as the gluten source.

Milling

All wheats were cleaned and tempered overnight to 16.5% moisture content. Wheats were milled following the procedure of Dexter et al. (1990) using a four-stand laboratory mill (Allis-Chalmers, Milwaukee, WI) in conjunction with a laboratory purifier (Black 1966) to produce semolina of approximately 66% extraction on a clean wheat basis. The milling area was controlled for temperature (21°C) and relative humidity (60%).

Glass Powder

Speriglass A3000 was kindly provided by Potters Industries (Valley Forge, Pa). The powder is made up of solid glass spheres having a mean particle size of 35 μm, with a particle size range similar to that of purified starch as can be seen in the SEM micrographs (Fig. 6.1).

Starch Preparation

The starch isolation procedure was based on the method of Morrison et al. (1984) with some modifications. Purification of the starch using a cesium chloride gradient was based on the work of South and Morrison (1990) and Sulaiman and Morrison (1990). Semolina (10g) was used in place of cracked kernels, eliminating the need for an acid presoak. A presoak in 0.5% sodium metabisulfite (Sigma, St. Louis, MO) for approximately 4 hours at 4°C was included to soften the protein matrix prior to enzymatic digestion. The solution was centrifuged at 4000 x g for 15 minutes and the supernatant discarded. The pellet was resuspended in a solution containing 5mg proteinase K (Roche Diagnostics, Mannheim, Germany) and was left to digest overnight in a 25°C shaking water bath. The solution was again centrifuged at 4000 x g for 15 minutes and the supernatant discarded. The pellet material was resuspended in a small amount of water and gently mashed through a 75 μm aperture sieve, washing with distilled water until the water ran clear.

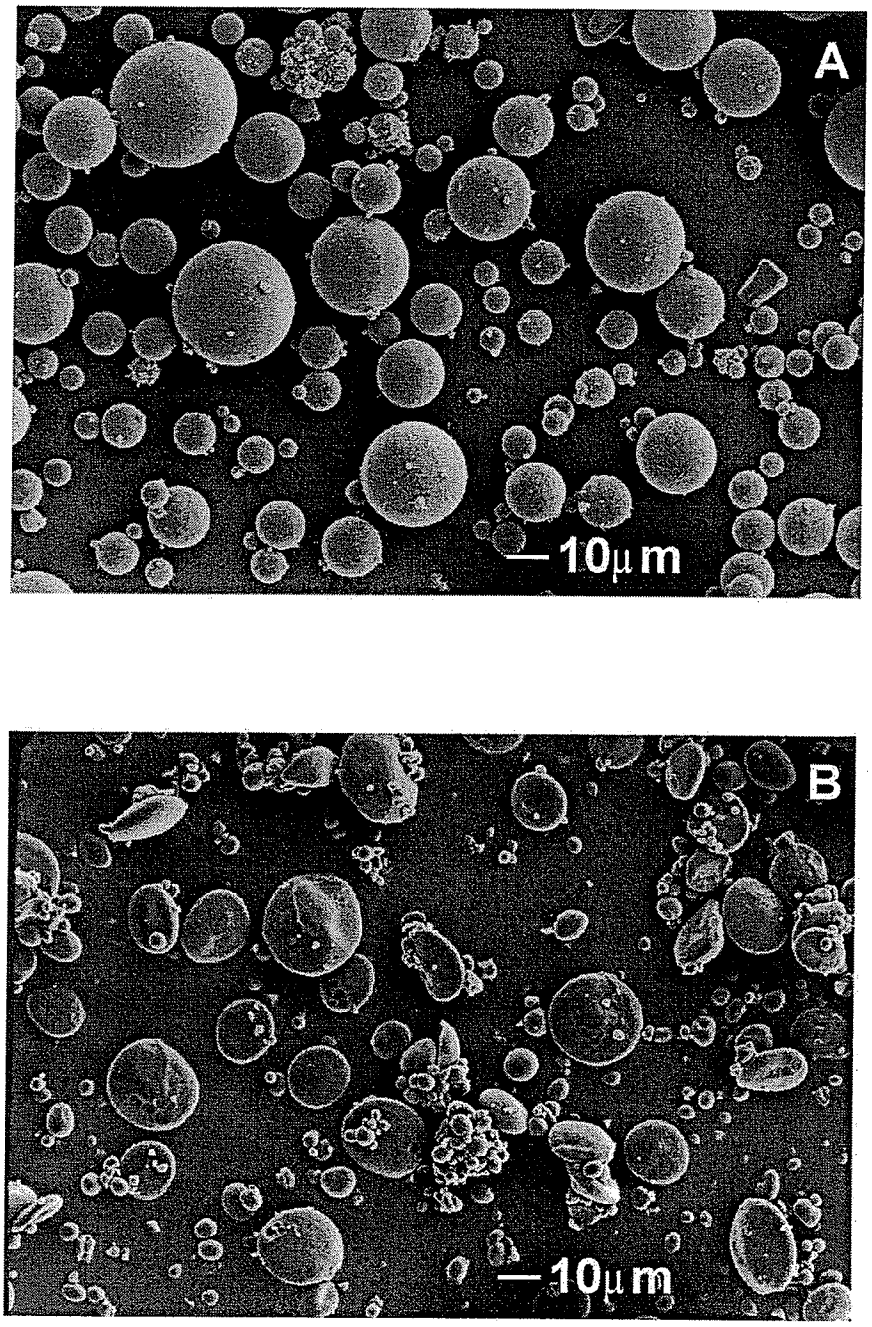

Figure 6.1. Scanning electron micrographs of A) glass powder and B) starch at magnification of 500x.

The resulting starch suspension was divided among 50 ml centrifuge tubes and centrifuged for 10 minutes at 28000 x g. The supernatant was discarded and the pellets combined using the minimal amount of water, followed by centrifugation at 28000 x g for 10 minutes, and the supernatant discarded. The pellet was resuspended in approximately 3 ml water, which was carefully layered over 20 ml 80% (w/v) cesium chloride (BDH, Toronto, ON) in 50 ml centrifuge tubes. Rinse water was added to the starch-water layer. The tubes were centrifuged for 30 minutes at 28000 x g and the cesium chloride was carefully removed. The pellets were washed 3 times with distilled water, centrifuging for 5 minutes at 28000 x g after each wash. The purified starch pellets were suspended in distilled water and collected on a 0.45 μm membrane filter (Millipore Corp., Bedford, MA) by suction filtration, rinsed with ethanol and air-dried to a moisture content of approximately 9%. Once dried, the starch was gently ground using a mortar and pestle. Starch from each variety was a composite of multiple isolation and purification procedures.

Surface Modifications

Heat-treated starch was prepared as described by Seguchi (1984) using purified starch isolated as described above. Prior to heat treatment, distilled water was added to the starch, which was then equilibrated for one week to bring the moisture content of the starch to 24.9%. The equilibrated starch was then heated for 60 minutes at 120°C in an open container. Final moisture content of the heat-treated starch after equilibrating in an open container at room temperature was 7.5%.

Glass powder and heat-treated starch were coated with bovine serum albumin (BSA) (BDH, Toronto, ON) by mixing the granules in a saturated solution of BSA for approximately 15 minutes. Excess liquid was removed by suction filtration using a Millipore apparatus (Millipore Corp., Bedford, MA) with a 0.45 μm membrane filter. The coated glass powder or starch granules were then air-dried, followed by gently grinding with a mortar and pestle to break apart aggregated material.

Particle Size Distribution

Starch or glass powder was suspended in a solution of 70% glycerol containing 0.1% KI/I_2. The suspension was placed on a microscope slide using a dropper, and a cover glass fixed in place. A minimum of 20 fields of view were captured, using a light microscope in tandem with a video camera connected to a computer and particle size frequency distribution curves generated (Fig. 6.2). Two aspect (vertical and horizontal) measurements made for each particle and the ratios calculated. Any particles with ratios that were less than 0.7 or greater than 1.4, indicating that the particle was either standing on its side (lenticular starch granules) or was fragmented, were eliminated from the particle size distribution calculations.

Gluten Isolation

Semolina was defatted with chloroform and air dried at room temperature. Gluten was extracted by initially mixing the defatted semolina with 0.001M NaCl to form a gluten

ball, then washing under a stream of distilled water to remove starch. The gluten ball was freeze-dried, then ground using a Krups 75 electric coffee grinder to form a fine powder.

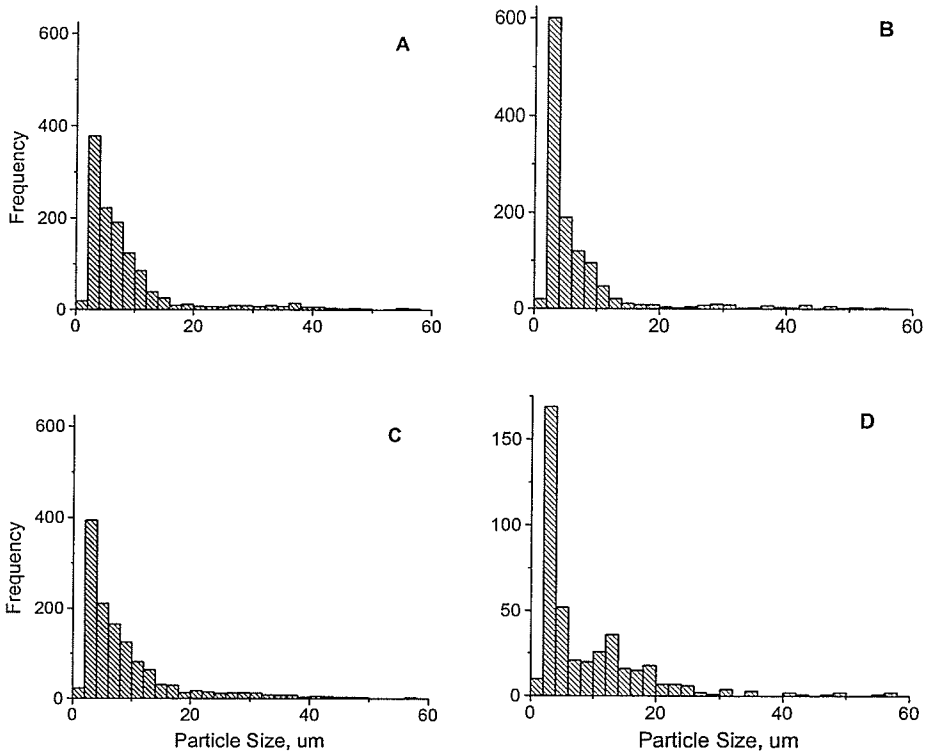

Figure 6.2. Frequency distribution of A) Durex, B) AC Morse, and C) Stewart 63 starch granules, and D) glass beads.

Analytical Tests

Moisture content was measured by single-stage air oven using AACC Approved Method 44-15a (AACC 2000). Protein content was determined by combustible nitrogen analysis (model FP-428, Leco Corp., St. Joseph, MI) calibrated against EDTA. Starch damage

was assessed using the Megazyme total starch assay procedure, AACC method 76-13 (AACC 2000). A 2g direct drive mixograph (National Manufacturing Division, TMCO, Lincoln, NE) was used to obtain mixing data with Mixsmart software supplied with the instrument.

Freezable Water Determination

The amount of free or freezable water was determined using a differential scanning calorimeter (DSC) model 2920 (TA Instruments, New Castle, DE) calibrated against an indium standard. Samples of dough prepared at a range of moisture levels (50-65% absorption) were transferred to sealed containers immediately after mixing. Sub-samples (approximately 10 to 15 mg) were removed and placed in hermetically sealed aluminum pans for analysis, and the weights recorded. Tap water was tested as a reference. The initial temperature of the DSC chamber was 20°C. The chamber was cooled at 10°C/min to a low of –60°C and held for 5 minutes so that the sample was fully frozen. The temperature was then ramped at 10°C/min to 50°C and the enthalpy (ΔH) of the endothermic transition recorded. Enthalpy was calculated as J/g based on total sample weight. All tests were done in triplicate and the average values reported. The amount of free or freezable water was determined by extrapolation of the curve generated from measurement of samples with a range of moisture contents to zero ΔH, where there is no freezable water.

Dough Preparation

Gluten-starch doughs (15% gluten: 85% starch) were prepared at 60% absorption using a 2g mixograph and mixed to 1 minute past peak. Glass powder was substituted for starch on a volume basis in order to account for considerable differences in density, replacing from 0% to 100% of the starch in the blend. Density of starch was taken to be 1.536 g/cm^3 (Rasper and deMan 1980). Density of glass powder was 2.5 g/cm^3 according to the supplier specifications. Substitution of starch with glass powder necessitated adjustment of the amount of water added to the mixture. Glass powder does not absorb water per se, but does hold it on the bead surface and in interstitial spaces between beads, and complete elimination of water proportional to the amount of glass powder resulted in a very dry dough and very erratic mixograph curves (data not shown). Absorption was therefore adjusted to 30% for the portion of the blend made up by glass powder to provide curves of similar mixograph consistency to those without any glass powder.

Rheology

Dynamic and creep measurements were made using a Rheometrics SR-500 fitted with serrated 25 mm parallel plates. A preset gap of 2.75 mm was used for all samples. Temperature was maintained at 25°C. Dynamic measurements were conducted at 0.35% strain, over a frequency range of 0.1 to 10 Hz. Creep compliance measurements were conducted within the linear regime at 20 Pa over 10000 seconds. The creep linear regime was determined as described by Edwards et al. (2001), by running a series of tests at

different levels of applied stress and identifying the linear region based on agreement of compliance curves. Once loaded in the rheometer each sample was carefully trimmed and the exposed edges coated with silicon grease. In addition, the rheometer was fitted with a humidity chamber to further prevent moisture loss during testing. All tests were conducted in duplicate and the average result reported. The overall coefficient of variation for dynamic testing was 4.0%, and for creep compliance was 9.1%.

Results and Discussion

Starch Granule Particle Size Distribution

The isolation procedure resulted in average recoveries of 62% starch from semolina, with a protein content of 0.28% ± 0.05. There was good representation of both large and small starch granules as can be seen in the particle size distribution histograms (Fig. 6.2). Visual assessment, by light microscopy, of the residue left after removal of starch indicated that a small amount of unextractable small granules remained embedded in the protein matrix (results not shown). The particle size distribution of starch granules was in general agreement with the results of other studies. Published findings included that A-type granules (10-35 μm) constituted more than 70% of the total weight, while B-type granules (10 μm or less) accounted for <30% by weight, but >90% of the total number of granules (Denegate and Meredith 1984, Evers and Lindley 1977, Morrison and Gadan

1987). It was also evident from the histograms (Fig. 6.2) and the SEM micrographs (Fig. 6.1) that the diameter of the glass beads fell within the same range as purified starch, and like starch the smallest beads represented the largest proportion.

Particle Size Effect

AC Morse starch had the greatest proportion of granules in the 0-10 μm range in comparison with the other two starch sources. It also demonstrated greater elastic character when used in gluten-starch dough, possessing higher G' and lower tan δ (Fig. 6.3) than starch from the other two cultivars. Ahmed and Jones (1990) cited several studies on filled synthetic polymers where an increase in modulus with decreasing particle size was found. Dough creep compliance also ranked in order of content of smaller granule (Fig. 6.3). Increasing the proportion of large starch granules has a sufficiently negative impact on gas cell wall stability of fermented doughs, disrupting the protein matrix, to result in coalescence of gas cells (Hayman et al. 1998). Lelievre and co-workers (1987) suggested that granule specific surface area affected the area over which gluten could spread thereby affecting crumb properties of bread made from gluten and starch. In other words, a larger proportion of small granules provides greater surface area, allowing for greater interaction with gluten with less disruption of the matrix. Our data appears to support the positive effect of small starch granules, but further studies using starch granules segregated by size would be useful in confirming this observation.

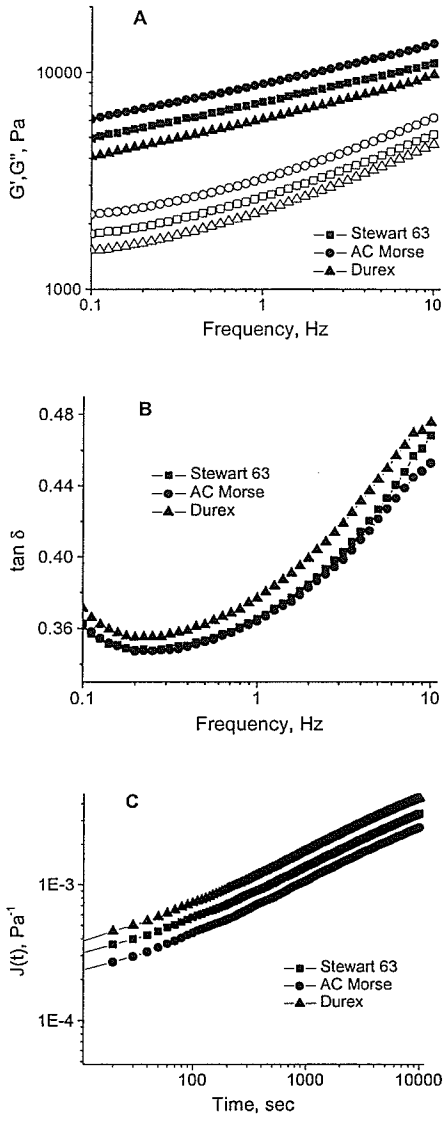

Figure 6.3. Effect of cultivar source of starch on A) dynamic moduli, closed symbols = G', open symbols = G", B) tan δ, and C) creep compliance of gluten-starch dough.

Glass Powder - Starch Interchange Experiments

The glass powder used in these experiments was assumed to be chemically inert and would therefore offer no means of surface interaction with protein or starch components

of the dough. Starch granules on the other hand exhibit hydrophilic surface characteristics (Seguchi 1984). One of the limitations that must be recognized in making comparisons between gluten-starch doughs and gluten-glass powder arises from the difference in shape of the filler particles, glass beads being spherical and starch granules being lenticular. Composite mechanical properties are known to be affected by size, shape and aspect ratio of the reinforcing particles (Ahmed and Jones 1990).

In order to confirm that cohesive dough could be produced when substituting starch with glass powder a series of blends were prepared ranging from gluten and 100% starch to gluten and 100% glass powder in a 2g mixograph, while adjusting the water absorption to produce a 'reasonable' mixing curve. Eliminating water proportional to the amount of glass powder resulted in very dry dough with erratic mixing curves, while maintaining 60% absorption for the entire sample weight of glass powder blends resulted in dough that was far too wet with very poor mixing quality (data not shown). Adjusting the water absorption to 30% for the glass powder proportion, while maintaining the gluten and starch proportion at 60% absorption produced acceptable mixing curves. Rasper and deMan (1980) found similar water retention levels (about 30%) for glass powder of similar particle size range. Mixing time to peak increased with increasing proportions of glass, but in all cases a cohesive dough sample was produced (Fig. 6.4).

In addition to assessing the mixing characteristics of the blends, freezable water was determined to establish that sufficient water was available to fully hydrate the gluten, particularly after substitution of starch with glass powder and adjustment of water

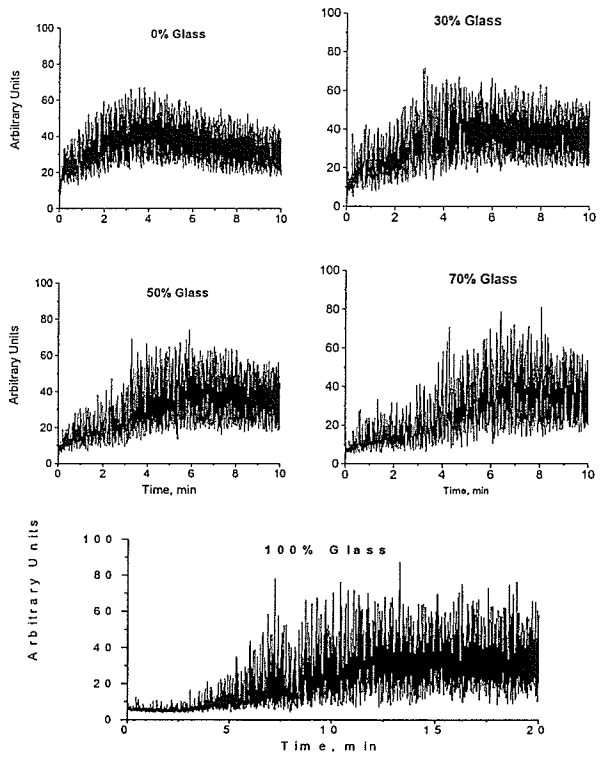

Figure 6.4. Mixograph curves of gluten-starch dough; gluten-starch-glass powder dough and gluten-glass powder dough. Percent glass represents the percentage of starch substituted with glass powder.

absorption levels. The range of absorption levels used (50 to 65%) was somewhat limited because the mixograph was unable to mix very dry stiff dough at very low moisture content and very high moisture content doughs were too sticky to handle and transfer to the DSC sample pans. The absorption levels used corresponded to 42.7- 47.9% dough moisture content. By extrapolating the curve to a ΔH of 0 J/g, freezable water in gluten-starch dough was observed to be available at >27% moisture content (Fig. 6.5). Using a regression line to reach a ΔH of 0 J/g lead indicated a lower dough moisture content, so the extrapolated line was used to err on the side of safety in terms of estimated the required moisture content for complete dough hydration. This value was intermediate to the 18% moisture content observed for hydrated gluten (Cherian and Chinachoti 1996)

and 33% moisture content for white bread crumb (Vodovotz et al. 1996). Dough moisture content was lowest when starch had been completely replaced by glass powder. Even in the glass-gluten dough ΔH was 54 J/g, well above 0 J/g, indicating that there was excess water in the system, so that more than sufficient water was available to fully hydrate the gluten. Therefore any differences observed in mechanical measurements of glass powder-gluten blends (with corresponding reductions in water absorption levels) were not attributable to insufficient gluten hydration and resultant dough stiffness.

Both G' and G" declined as starch was replaced up to 30% with glass powder, then remained essentially unchanged to 50% substitution (Fig. 6.6). At glass powder proportions greater than 50% the moduli started to increase, exceeding the gluten-starch control once all of the starch was replaced with glass powder. Except at low frequencies, tan δ increased with increasing proportion of glass powder (Fig. 6.6C). As starch was replaced with glass powder from 0% up to about 50%, the total surface area over which there was adhesion between matrix and filler decreased, resulting in an increasing proportion of energy dissipation at the gluten-filler (starch/glass powder) interface relative to energy stored. Edie and co-workers (1991) stated that in composite materials consisting of elastic fillers embedded in a viscoelastic matrix, in their case carbon fibers and polymer resin, that deformation energy is mainly dissipated in the matrix and at the matrix-filler interface. They demonstrated that increasing the volume fraction of a filler that adhered poorly to the matrix resulted in substantial increases in dissipation attributable to the filler-matrix interface. The threshold at which bulk rheological properties of the filler exerted a stronger influence than the filler-matrix interface was

between 30% and 50% substitution with glass powder. At substitution levels in excess of 50% glass powder, increasing tan δ resulted from increasing friction between the rigid glass beads leading to dissipation of energy as heat.

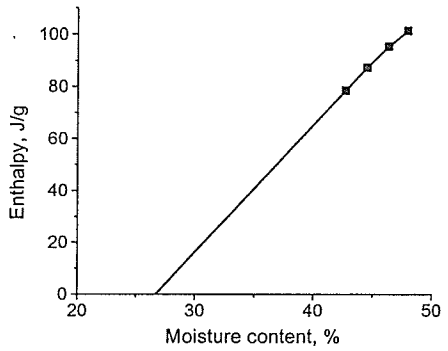

Figure 6.5. Dough freezable water content determined by extrapolation of a series of gluten-starch dough samples of known moisture content and measured ΔH.

Creep compliance exhibited similar behaviour to the dynamic measurements (Fig. 6.7). Compliance, J(t), increased to a maximum at 30% substitution with glass powder. Increasing compliance of durum semolina dough has been related to decreasing overall dough strength (Edwards et al. 2001). At 50% substitution J(t) was slightly lower than at 30%, but the trajectory of the curve at longer times began to flatten off (Fig. 6.7). By 70% substitution J(t) trajectory was much flatter and dropped below the gluten-starch control dough after approximately 1000 s. Once all the starch had been replaced with glass powder, J(t) was much lower than the gluten-starch control dough and exhibited the flat trajectory seen at 70% substitution. Flattening off of the compliance curve at 50% substitution and higher indicated a transition to a more solid-like system, a phenomenon also observed by Rayment et al. (1998) with increasing volume fraction of rice starch filler in a guar galactomannan entanglement solution.

The simplest possible case for describing the modulus (E) of a two-phase composite material is given by Ahmed and Jones (1990):

$$E_c = E_p V_p + E_m V_m \quad (1)$$

where the subscript c represents the composite, p the particulate filler and m the matrix. V represents the relative volume fraction. Glass beads are more rigid in comparison with starch granules, and therefore according to equation (1) dough should become stiffer as glass powder makes up increasing proportions of the particulate filler. This behaviour has been demonstrated in filled polymer systems, using polymers of varying molecular weight with spherical glass beads as the filler (Walberer and McHugh 2001). That, however, was not the case at the lower volume fractions of glass powder in dough blends.

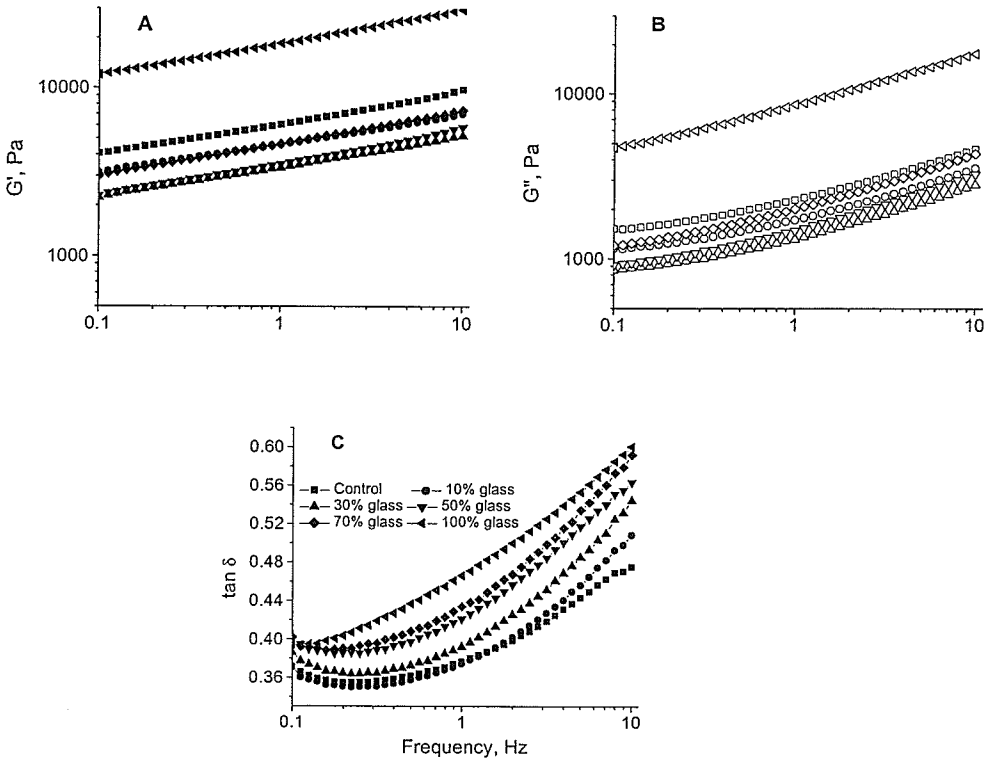

Figure 6.6. Dynamic measurements made on gluten-starch doughs where glass powder replaced from 0 to 100% of the starch. A) Storage modulus, G' B) Loss modulus, G" and C) tan δ

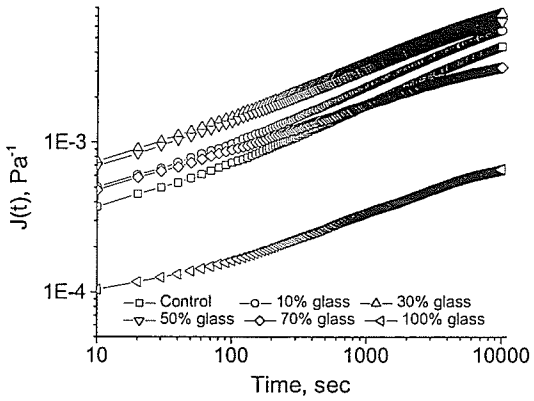

Figure 6.7. Creep compliance measurements made on gluten-starch doughs where glass powder replaced from 0 to 100% of the starch.

Depending on the nature of the matrix and the filler, van Vliet (1988) identified two extremes in composite behaviour. In the first case, where there is a strong interaction between the filler and matrix, under conditions of small deformation there would be an increase in modulus of a gel with increasing volume fraction of filler, provided that the filler is stiffer than the gel. At the opposite extreme, where there is no interaction between matrix and filler, there would be a decrease in modulus with increasing volume fraction of filler under small deformation test conditions. These cases hold true only at relatively small volume fractions. Substitution of starch with glass powder up to approximately 50% resulted in behaviour similar to the second case: a decrease in modulus. G' declined to a greater extent relative to G", resulting in increased tan δ. Two conclusions may be drawn from these data: a) glass powder did not interact with the protein matrix, and b) starch granules were interacting with the protein matrix in order for there to have been a decrease in modulus and increased tan δ, and an increase in compliance upon its replacement. In excess of 50% substitution of starch by glass powder, the bulk rheological properties of the glass filler exerted greater influence on the moduli and the

dough became stiffer due to the rigidity of the glass beads and the frictional contacts between them, the latter of which was clearly evident at 100% substitution.

Filler Surface Modifications

In order to investigate the effects of surface bonding further, glass powder was coated with BSA to provide H bonding sites in the gluten-glass powder interface to compare its mechanical properties with those of gluten-non-coated glass powder dough. Completely replacing the glass powder with BSA-coated glass powder resulted in reduced dynamic moduli (Fig. 6.8a), but increased tan δ (Fig. 6.8b), and increased creep compliance (Fig. 6.8c). Tests performed on a gluten-glass powder dough using a 70:30 blend of non-coated glass powder and BSA-coated glass powder, respectively, resulted in dynamic moduli and J(t) that were intermediate to those of non-coated and BSA-coated glass powder. In contrast, tan δ of the blend was similar to the dough made with BSA-coated glass powder (Fig. 6.8b). BSA (10 mg) simply added to the gluten-glass powder dough had no effect on either dynamic measurements or on J(t) (Fig. 6.8). The amount of free BSA (10 mg) represents approximately the amount of protein that was found on the coated glass beads (1.2% protein) in the 70:30 blend. Providing H bonding sites by coating the glass powder with BSA altered the dough rheological properties so as to behave closer to what would be expected for gluten-starch dough. The BSA coating allowed the beads to slide across each other more easily than could the non-coated beads, with an accompanying decline in moduli, particularly G' (Fig. 6.8a). Therefore, tan δ values were greater than uncoated glass powder on its own or with free BSA (Fig. 6.8b).

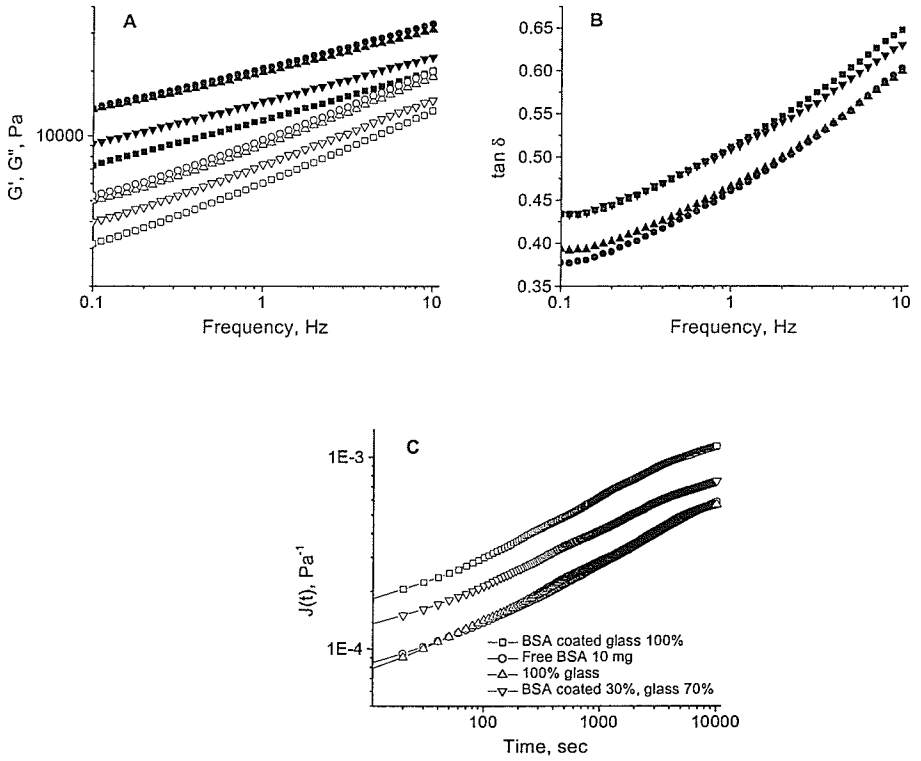

Figure 6.8. Effect of BSA coating of glass powder compared with non-coated glass powder used in gluten-glass powder doughs, and a 30% BSA coated / 70% non-coated glass powder-gluten blend dough. A) dynamic moduli, closed symbols = G', open symbols = G". B) tan δ. C) creep compliance.

Heat-treatment of starch granules alters their normally hydrophilic surface to hydrophobic (Seguchi 1984). Hoover and Vasanthan (1994) noted that heat-moisture treatment of wheat starch caused the crystalline arrays to become more closely packed and ordered, resulting in decreased susceptibility to α-amylase and acid hydrolysis and decreased granule swelling leading to greater amylograph viscosity. In the current study, dough prepared from gluten and heat-treated starch had considerably greater elastic character than the gluten-starch control dough, with higher dynamic moduli (Fig. 6.9a), lower tan δ (Fig. 6.9b) and lower J(t) (Fig. 6.9c). Hydrophobic surface characteristics of the heat-treated starch would have increased its affinity to hydrophobic areas of the

gluten protein. Larsson and Eliasson (1997) also suggested the possibility of hydrophobic interactions when they observed increases in moduli with addition of heat-treated starch to wheat flour. It could be argued that heat treatment of starch could increase the rigidity of the granules or alter their shape or size as a result of partial gelatinization if there was sufficient water in localized areas. To alleviate these concerns DSC testing was conducted on the heat-treated starch. No evidence of gelatinization was observed (data not shown).

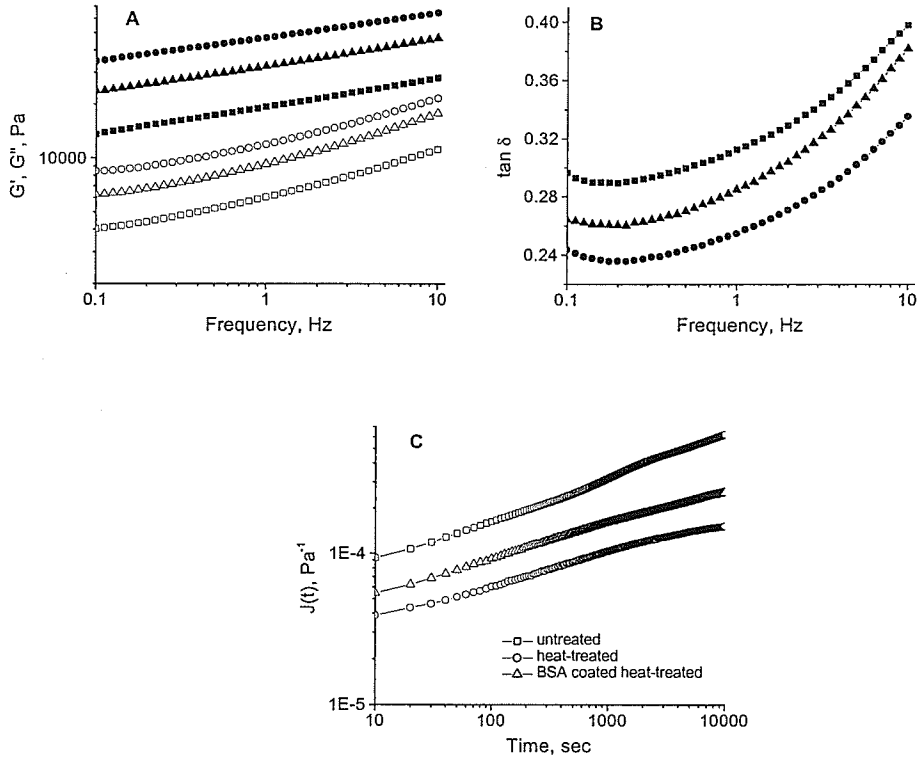

Figure 6.9. Comparisons of gluten-starch doughs prepared from untreated (hydrophilic) and heat-treated (hydrophobic) starch, and BSA-coated heat-treated starch. A) dynamic moduli, closed symbols = G', open symbols = G". B) tan δ. C) creep compliance.

To confirm the importance of surface interactions at the starch granule-protein interface, heat-treated starch was coated with BSA in the same manner as the glass powder. Coating of the starch granules increased the protein content of the starch from 0.25% to 1.89%. Masking of hydrophobic sites by coating with BSA reduced starch granule surface interactions with the gluten protein, with dynamic moduli, tan δ and J(t) that were in all cases intermediate to gluten-starch control dough and gluten-heat-treated starch dough (Fig. 6.9). Altering the bonding sites in the interface region dramatically altered the linear viscoelastic properties of the gluten-starch model system, confirming the importance of the starch granule surface characteristics and the nature of protein-starch bonding on durum dough linear viscoelastic behaviour.

Conclusions

Starch does contribute measurably to durum dough rheological properties. Increased proportions of smaller granules increased dough elastic character, similar to behaviour observed in filled polymers (Ahmed and Jones 1990). Substitution of starch with glass powder confirmed that granule surface properties were involved in interactions with gluten protein, and that the surface of the glass beads was inert. At volume fractions of less than 50% there was an increase in J(t) and a reduction in dynamic moduli. G' declined more than G" resulting in increasing tan δ, which was attributable to lowering of overall adhesion of the filler particles to the gluten matrix. In excess of approximately 50% substitution, rigidity of the glass beads and friction between beads exerted a greater

influence than the gluten-filler (starch/glass powder) interface in determining the linear viscoelastic properties of the dough. Using BSA to provide H bonding sites on the glass beads reduced dynamic moduli and increased J(t) relative to non-coated beads. Coating glass beads with BSA increased the ability of the coated beads to slide across each other compared to non-coated beads. Heat treating starch to generate a hydrophobic surface, as opposed to the normally hydrophilic starch granule surface, led to increased interaction between starch granule surfaces and gluten. Hydrophobic interactions between heat-treated starch and gluten had greater stability, and greater ability to store energy than the weaker H bonds predominant in the interface between native starch and gluten. Masking of hydrophobic sites by coating heat-treated starch with BSA reduced surface interaction, confirming the importance of starch granule surface properties in determining dough viscoelastic behaviour.

CHAPTER 7

General Discussion

Durum dough may be processed under a variety of conditions, including slow or high speed mixing, extrusion, high-speed extrusion, sheeting and cutting, and in some instances it is processed into bread. The challenge is to understand the physicochemical basis of dough rheology that controls dough flow and deformation so as to be able to predict dough behaviour under a range of processing conditions.

Dough Fundamental Rheological Measurements

Fundamental rheological measurements provide tools to advance the understanding and solution of complex dough processing concepts and problems. Several techniques have been employed for investigating the fundamental mechanical properties of dough. These include stress relaxation (Launay and Buré 1974, Rao et al 2000, 2001), creep and creep recovery (Campos et al 1997, Edwards et al 2001, Hibberd and Parker 1979), and dynamic oscillatory measurements (Abdelrahman and Spies 1986, Amemiya and Menjivar 1992, Edwards et al 1999, Faubion and Hoseney 1990).

Dough may be viewed as a heterogeneous composite material, composed of the gluten matrix with embedded starch granules, along with several minor components including lipids, minerals and non-starch polysaccharides. Assuming insignificant contribution by

the minor components, the properties of the composite depend mainly on the bulk rheological properties of the gluten matrix and starch filler. The properties of the composite are also influenced by the total volume fraction occupied by the starch granules and interaction between granules, and the degree of adhesion and interaction between the starch granules and protein matrix (Ahmed and Jones 1990). Fillers are considered either reinforcing or non-reinforcing depending on whether or not there is adhesion between the matrix and filler (Edie et al 1991).

In my study a gluten-starch model system was used to study the role of starch and the starch-gluten interface in durum dough viscoelastic behaviour. Starch was substituted with increasing volume fractions of glass powder. Beads making up the glass powder are rigid, having higher elastic modulus than starch granules. Therefore, upon replacement of starch granules with glass powder the expectation was that the elastic modulus of the composite would increase. That was not the case at volume fractions of less than 70% replacement of the starch. Glass powder has an inert surface and, therefore, did not adhere to the gluten matrix, and a decline was noted in both G' and G" with increasing proportions of glass powder. The decline in moduli infers there was a reduction in degree of adhesion between filler (starch/glass powder) and matrix, leading to the conclusion that starch granules adhere to or interact with gluten, acting as reinforcing filler particles. When the volume fraction exceeded 50% glass powder, bulk rheological properties of the glass powder began to exert a stronger influence than the filler-matrix interface interactions, and the moduli increased, exceeding the starch-gluten control at 100% substitution. Starch granules act as reinforcing filler particles

through their interaction at the gluten-starch interface and do not, on their own, contribute to durum dough viscoelastic properties. It is, therefore, important to examine the nature of the gluten matrix itself and the types of interactions that can occur within the matrix and at the matrix-filler interface.

Oscillatory tests were found capable of discriminating durum doughs of varying strength, but tan δ was strongly influenced by protein content (Edwards et al 1999). Therefore, dynamic tests should be limited to studying samples of similar protein content. Dynamic measurements do not provide information on molecular orientation or processes. This study showed that compliance testing provided more revealing information than dynamic measurements on the molecular basis of dough strength through modeling of the compliance curve to separate different time dependent molecular processes. Use of the equation defining the six element Burgers model (refer to Chapter 4) to describe creep compliance demonstrates that the time-dependent molecular processes are additive in compliance functions. Retardation spectra provided clues as to a probable source for fundamental differences in durum versus common wheat doughs that affected their extensibility and viscosity properties. Differences between wheat classes were clearly evident for the longer retardation times. Greater extensigraph strength of common wheat doughs was associated with higher retardation strength for the longer retardation times, and higher viscosities (Edwards et al 2001). Durum doughs exhibited exactly the opposite behaviour, with stronger durum doughs showing the lowest retardation strengths for the longer retardation times, but also the highest viscosities (Edwards et al 2001). This behaviour of the durum doughs suggests

there was a stronger influence of physical crosslinks on linear viscoelastic properties (higher steady state viscosities for lower molecular weights) as discussed by Tanaka and Edwards (1992a,b,c) for reversible physical gels in the unentangled regime. Higher steady state viscosity and relative lack of extensibility suggests that durum dough strength is a function of the density of crosslinks present. Increased retardation strength at long times and higher steady state viscosities for strong common wheat doughs suggests that the larger polypeptides played a greater role in determining dough strength. Common wheat dough behaviour was more similar to a physical gel containing both entanglements and crosslinks, but where entanglements dominate. In other words, it appears that the balance between dough strength and extensibility is dependent on the relative distribution of long chains to short chains and their underlying molecular mobilities. A unique aspect of this study for understanding dough rheology was the analogy of gluten behaviour in the dough with that of a reversible physical gel with crosslinks and entanglements.

Molecular Weight Distribution

Durum wheat glutenin isolated from Durex (strong) and AC Morse (intermediate strength) and added to base semolina ranked the samples according to the donor cultivar dough strength using dynamic and creep measurements. Interestingly, under the same test conditions, the respective HMW-GS and LMW-GS isolated from both cultivars did not demonstrate dough strength differences between each other when added on a constant protein basis. The obvious conclusion would be that there are differences in the

relative distribution of the long chains and short chains in the unfractionated glutenin from the two cultivars that resulted in different compliance responses, underlining the importance of molecular weight distribution to dough linear viscoelastic properties.

HMW-GS and LMW-GS both contribute to dough strength. Glutenin subunit rheological behaviour is affected primarily by H bonding, disulfide bonds and by molecular weight distribution in combination. Since the importance of molecular weight distribution has already been explained, the glutenin subunits provide good examples for explaining the roles of bond types. The cultivars used here possessed different HMW-GS patterns (6+8 and 7+8). Based on this study, on an equivalent protein content basis there is not an advantage to having one HMW-GS type over the other in durum dough. This is in contrast to published studies that claim certain HMW-GS confer greater dough strength than others (Boggini and Pogna 1989, duCros 1987, Ruiz and Carrillo 1995), although there is disagreement among these studies as to the relative rankings of the various types. These studies were all based on correlative analyses and therefore did not provide a direct cause and effect relationship.

The source of LMW-GS did affect creep compliance response. Enrichment with LMW-GS from the strong and intermediate strength cultivars (Durex and AC Morse) reduced compliance equally and to a greater extent than with enrichment from the weakest cultivar (Stewart 63). In addition, LMW-GS from both of the stronger durum cultivars reduced creep compliance to a greater extent than did an equivalent addition of HMW-GS. Durex and AC Morse possess LMW-GS designated as LMW-2, which are

associated with superior pasta quality. Stewart 63, on the other hand, possesses LMW-1 that are associated with poor pasta making quality. LMW-2 are generally expressed in greater amounts than LMW-1. It has been suggested that the improved quality of dough possessing LMW-2 is primarily a function of greater relative quantity (Autran et al 1987, D'Ovidio et al 1999, Masci et al 1995). The results of this study refute that hypothesis, since the mechanical performance of dough enriched with LMW-GS from all three cultivars should have responded equally when added in equal amounts, which was not the case.

The Role of Disulfide Bonds

The density of initial elastically effective crosslinks is considered to largely determine the instantaneous compliance of dough upon application of a stress (Bloksma 1975). Disulfide bonds are the most likely source of initial elastically effective crosslinks. Instantaneous compliance of doughs enriched with HMW-GS was higher than when enriched with LMW-GS. Longer chain lengths separate disulfide bonds (at the C and N termini) in HMW-GS than in the shorter chain length LMW-GS. Therefore, the density of disulfide bond crosslinks would be less for HMW-GS than LMW-GS for a given quantity of protein. Additionally, the distance before reaching maximum chain extension with applied stress would be greater for HMW-GS than for LMW-GS, assuming the presence of cysteine amino acids at both ends of individual subunits (Shewry et al 1999). These two characteristics combined contributed to the higher overall compliance of HMW-GS enriched doughs, but in particular to their higher

instantaneous compliance. The sole exception was dough enriched with Stewart 63 LMW-GS, which as discussed in Chapter 5, had a lower density of crosslinks for a given protein content than the other two cultivars, resulting in even higher overall compliance than those containing HMW-GS.

At long times, when steady state flow was reached, the disulfide bonds contributed to viscous flow through rearrangement of the network via breaking and reforming of bonds, a concept originally proposed by Bloksma (1975). The steady state viscosities of HMW-GS and LMW-GS, each added at levels of 20 mg, were approximately half that of their respective unfractionated glutenin, added at 40 mg for AC Morse and Durex. The effect of HMW-GS and LMW-GS on dough viscosity appears to be additive.

The Role of Hydrogen Bonds

The LMW-2 types conferred greater strength than the LMW-1 type on a constant protein basis, resulting from differences in the density of reversible physical crosslinks. Sources of transient reversible crosslinks, in addition to disulfide bonds, may include hydrogen bonds, ionic bonds and hydrophobic interactions (Tanaka and Edwards 1992a,b,c). D'Ovidio and co-workers (1999) sequenced the 42k band found in LMW-2 (but absent in LMW-1) and found the repetitive domain contained a high proportion of glutamine, with the repeat regions having the consensus sequence PPFSQQQQ. The calculated proportion of glutamine was 34.6%, providing surfaces rich in H bonding sites capable of forming associations with water and with other glutenin chains. Belton

(1999) proposed that common wheat HMW-GS interact with each other by a combination of disulfide bonds and hydrogen bonds, and that H bonds are responsible for gluten elasticity in a hydrated dough. Because of the large number of interchain H bonds it is highly unlikely that all would break simultaneously, but at any given time there would be bonded regions and unbonded mobile regions. Belton (1999) suggested that hydrated loops form in adjacent chains, with interchain H bonds forming between loops. When a stress is applied the loops deform first until the network is stretched to the point where H bonds pull apart and the protein strands slide along each other. The network relaxes by returning to the equilibrium of loops intervened by interchain H bonds, with the driving force for restoration of molecular structure consisting of the preferred conformational entropy of the loops and the enthalpy of the interchain H bonds. It is highly probable that a similar train and loop process occurs in the LMW-GS of durum wheat. Following instantaneous compliance, there is a period of retarded elastic compliance where the network undergoes configurational rearrangement, aligning along the direction of applied shear, before finally reaching steady state viscous flow. To accommodate that rearrangement it is probable that the network is undergoing the train and loop process of breaking and reforming H bonds, until the point of viscous flow where the disulfide bonds are fully extended and begin to break under the applied stress. The presence of the protein associated with the 42k band in LMW-2 would impart a greater density of disulfide bonds (cysteine at either end of the 42k subunit) and hydrogen bonds because of the hydrophilic repetitive domain, than would LMW-1 which lacks this subunit. The greater density of crosslinks for a given amount of protein would provide greater resistance to configurational rearrangement of

the original network and less viscous flow, accounting for the strength differences seen between LMW-1 and LMW-2.

In addition to interchain H bonds within the protein structure, H bonds at the starch granule-gluten interface contributed to durum dough linear viscoelastic properties. Altering the surface properties of the filler component (starch with a hydrophilic surface) either by substitution with glass powder (inert surface) or by heat-treating the starch (hydrophobic surface) altered the degree of adhesion between the filler and the hydrated gluten protein, affecting the resulting rheological behaviour of the dough. Removing the source of H bonds by substitution of starch with relatively small volume fractions of glass powder (with its inert surface), effectively reduced the interaction between filler and matrix, leading to a decrease in moduli and an increase in compliance, as predicted by van Vliet (1988). Conversely, substituting native starch with heat-treated starch led to greater adhesion between the filler and matrix via hydrophobic interactions, increasing moduli and reducing compliance. Larsson and Eliasson (1997) also found that adding heat-treated starch to flour increased moduli. Masking some of the hydrophobic surface by coating the heat-treated starch granules with BSA reduced the degree of interaction between filler and matrix, thus reducing moduli and increasing compliance, confirming the importance of the surface characteristics of the starch granule and their role in durum dough linear viscoelastic behaviour.

Future Studies

After completing the writing of the research papers included in this thesis, several additional questions come to mind regarding dough viscoelastic properties. The parameters designated by each element of the six-element Burgers used to describe creep compliance represent different, but additive molecular processes. To relate the various parameters, whose magnitudes are related to the gluten strength of the cultivar, to pasta processing characteristics, it would be of value to use extrusion equipment capable of monitoring or measuring rheological behaviour of pasta dough during processing. It seems logical that dough with high steady state viscosity and low overall compliance would exert greater back pressure during extrusion and experience greater die swell post-extrusion than would a weaker dough when processed under comparable conditions. The ability to sample dough throughout the extrusion process and characterize its molecular structure and rheological properties would allow one to determine if there are differences in the gluten development process among doughs differing in fundamental rheological properties.

It appears, from preliminary work comparing durum and common wheat doughs, that different mechanisms are involved in determining viscoelastic properties. Although both entanglements and crosslinks are probably present, durum dough properties are influenced mainly by reversible physical crosslinks. In contrast, common wheat dough properties, including greater extensibility than durum doughs, appear to be more the result of entanglements, due to the greater concentration of the longer chain HMW-GS.

Future work using a similar approach to that used for elucidation of durum dough viscoelastic properties, gluten fractionation and enrichment studies with the fractions, would be beneficial in establishing the mechanisms controlling common wheat dough viscoelastic behaviour.

The differences between common wheat and durum wheat may be the result of differences in molecular weight distribution within the glutenin component. Performing enrichment studies with HMW-GS and LMW-GS fractions blended in various ratios to alter molecular weight distribution would provide additional information on the role of molecular weight distribution in the glutenin component. As well, one could verify that the contributions of each fraction to dough viscosity are additive. Preparing blends that contain glutenin fractions from different classes of wheat, for example, durum LMW-GS with Canada western extra strong HMW-GS, provides an additional avenue for evaluating the role of each fraction, and its source in determining dough viscoelastic properties. Another means of corroborating the importance of molecular weight distribution would involve the use of durum lines that have been crossed with common wheat to incorporate some of the D genome material encoding for long chain HMW-GS, and doing comparisons between the parent lines and their transgenic progeny. In addition to comparing the empirical measurements and compliance model parameters, it would be interesting to generate retardation spectra of the parents and the durum wheat progeny possessing common wheat HMW-GS to see if there are different mechanisms at play affecting the secondary retardation times and their intensities.

Starch interactions with gluten were shown to affect dough rheological properties. Models could be developed to predict and quantify the extent of the contribution of starch granules, based on size distribution and surface characteristics. The effects exerted by particle size should be studied with isolated populations of large and small granules to measure their contributions individually, and to establish if the contributions are additive based on granule size distribution. In addition, prediction models could be established by conducting substitution experiments using glass powder that had been fractionated based on size. Sequential substitution of starch with glass powder could be conducted with relatively narrow intervals, for example at increments of 5%, up to 50% glass powder. The maximum range in moduli and compliance between the starch-gluten control and starch/glass powder-gluten was evident at 50% substitution. Exceeding 50% glass powder, bulk rheological properties of the glass beads begin to exert a stronger influence than the filler-matrix interface interactions. Surface characteristics could be studied by controlled modification of starch granule surface properties, for example by esterification, which reduces the tendency for H bonding. After determining the degree of modification, substitution experiments could be run and the changes in rheological response measured. Knowing the degree of modification would allow calculation of interface interactions, and a mathematical model could be developed. It is probable that knowledge of both the roles of particle size and surface characteristics would be required to develop a realistic model.

The work that makes up the body of this thesis answers some of the questions surrounding the viscoelastic behaviour of durum dough, and leads to several new areas

of inquiry. Methods were developed that measure dough linear viscoelastic properties and discriminate differences in dough strength. Hypotheses were presented to explain the mechanisms involved in durum dough viscoelasticity, including the role of reversible physical crosslinks in gluten strength, the importance of molecular weight distribution within the glutenin component, and the role of starch granule surface characteristics in starch-gluten surface interactions.

CHAPTER 8

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