Basic Rheology of Bread Dough with Modified Protein Content and Gluten-in-to-Gliadin Ratios

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ABSTRACT

Dough measurements, as determined on equipment such as the mixograph, farinograph, extensigraph and alveograph, all utilize arbitrary units that cannot readily be converted to more useful scientific dimensions (Levine 1987). Basic rheological instruments are capable of providing the essential, or fundamental, details of the material’s rheological properties, unlike empirical tests. Rheological properties can be considered as a continuum between two ideal states, those of pure elasticity and of pure viscosity. A purely elastic rubber band does not flow but snaps back to its starting position, while purely viscous water flows without recovery. Most materials, including bread dough, demonstrate behavior that is a combination of both states and basic rheometry can elucidate and quantify these properties.

Wheat dough is a unique material formed when wheat flour is mixed with water creating a viscoelastic dough that retains gas (Walker and Hazelton 1996). The rheological characterization of wheat flour dough is essential to produce information concerning the quality of the raw material and the textural characteristics of the finished product. Dough studies in which the basic principles of physics have been applied have involved basic rheological measurements of shear stress in steady shear, creep, stress relaxation and extension (Blokasma and Bushuk 1988, Janssen et al 1996a, Safari-Ardi and Phan-Thien 1998). These studies have had the aim of completely characterizing dough and of finding reliable rheological tests that can differentiate dough types. Strain sweep experiments and stress-relaxation tests on Australian strong flour indicated that tests at higher shear strains can differentiate flour types (Phan-Thien and Safari-Ardi 1998, Safari-Ardi et al 1998).

A study on the effects of starch-protein interaction on rheological properties has also been performed on two South Australian wheat cultivars where a synthetic aqueous mixture of dough was used (Chiruta et al 1997). Khatkar et al (1995) and Janssen et al (1996a) studied the effect of varying the glutenin-to-gliadin ratio on rheological property of gluten dough and showed that glutenin contributed to elastic and gliadin to the viscous property of hydrated gluten. Though it has been shown that basic rheological tests can differentiate flour samples, very little work of this nature has been reported so far on the relationship between different protein quantities, different glutenin-to-gliadin ratios, and their functional properties.

The objective of this study was to use basic rheological tests (elongational and shear viscometry) to separate the effects of protein quantity and composition on dough properties.

MATERIALS AND METHODS

Samples

Wheat flours Banks, Hartog, Rosella, and Sunbri were obtained from BRI Australia Ltd., North Ryde, NSW, for the study. The high molecular weight gluten subunit (HMW-GS) composition of the flours is given in Table I.

The flour components (starch, gluten, glutenin-rich and gliadin-rich fractions) for enrichment studies were prepared as described by MacRitchie (1987) and Uthayakumaran et al (1999). The nitrogen content of the components was determined by the Dumas total combustion method using an elemental analyzer (CHN-1000, Leco Inc., St. Joseph, MI). Protein (%) was estimated as N × 5.7.

Altering Protein Content of Flour at Constant Glutenin-to-Gliadin Ratio

Blends (10.0 g) of each of the base flours, using gluten and starch isolated from that flour, were prepared as previously described (Uthayakumaran et al 1999). Based on the protein content of each flour, gluten and starch blends of flour and gluten isolated from it were prepared to have 110, 120, and 130% of the protein content in the base flour (increasing protein). Formulations containing 80 and 90% of the protein of the parent flour were prepared by blending the flour with starch isolated from that flour (to dilute the protein).

Altering Glutenin-to-Gliadin Ratio at Constant Protein Content

Gluten, glutenin, or gliadin prepared from the parent flour were added to the flour to vary the glutenin-to-gliadin ratio while keeping the protein content constant at 120% of the protein content of the parent flour.

Measurement of Functional Properties

The amounts of water to be added were calculated from the protein content and the moisture of the blend using the standard method (AACC 2000). Blend, water, and salt solution (6.67%...
w/v) were mixed in a 10-g mixograph to peak dough development and rheological measurements were then made. Mixing was done in triplicate for each sample, and the mean peak dough development time was calculated from the mixing curve (Gras et al 1990).

Two rheological tests were selected to provide information on two types of rheological flow: elongation and shear. Elongational flow is thought to be the predominant type of flow occurring in the dough surrounding the inflating gas bubbles during fermentation and baking (van Vliet et al 1992, 1993). Shear flow is the predominant flow experienced during dough mixing and was investigated by applying a constant rate of shear (shear viscometry) to the sample. Both the elongation and shear viscometry impose high strain levels to the dough, deforming the samples until they are physically broken. With elongational tests, the dough sample ruptures, and with viscometry, the dough undergoes edge fracture at high strains. High strain rheology was selected in light of previous findings that small strain rheology is unable to differentiate between functionally very different flours (Safari-Ardi and Phan-Thien 1998). All basic rheological measurements were conducted in triplicate.

Elongational Testing
The elongational properties of the doughs were studied using a constant-strain-rate extension test performed on a Universal Testing Machine (model SST-5000, United Calibration Corp., Huntington Beach, CA). The dough was compressed between a fixed and a moving upper grip both with a diameter of 30 mm and both plates were lined with sandpaper to enable adhesion to the plates. The dough sample was then rested for 45 min (for complete stress relaxation) before testing. Moisture loss was prevented by applying a layer of food-grade petroleum jelly (free of ethanol residue) around the edge of the sample. After the 45-min rest, the dough was pulled apart at an exponentially increasing speed to maintain a constant strain-rate (0.01/sec) in the dough sample (Fig. 1). Control of the top plate speed and collection of data were performed by a desktop computer running a program written in QuickBasic version 1.1 (Microsoft Corporation, Seattle, WA, 1992). Force and distance data collected by the computer were used to calculate the rheological parameters of strain and elongational viscosity (Pa-s). Strain, defined here as Hencky strain, was calculated using

\[ \varepsilon = \ln \left( \frac{l}{l_0} \right), \]

where \( l_0 \) is the original length of the sample, that is, the initial plate separation (5 mm). The stress is \( \sigma = F/A \), where \( F \) is the force exerted by the sample on the load cell, and \( A \) is the minimum cross-sectional area of the sample (usually at the midpoint of the sample). Preliminary investigations showed that at strains >1, the elongated dough sample took the shape of a cylinder (Fig. 1B and C). The minimum cross-sectional area of the dough sample was calculated, assuming that during elongation the volume of dough sample did not change and was cylindrical in shape. The extensional viscosity is \( \eta_E = \sigma / \varepsilon \) where \( \varepsilon \) is the strain rate in the sample. The tests were performed in an air-conditioned laboratory with a variation of ±0.5°C in the 24°C ambient temperature.

Viscosity Testing
The shear properties of the wheat doughs were studied using shear viscometry. The mixed dough was mounted on a controlled stress rheometer (Stresstech, Reologica Instruments AB, Lund, Sweden) in the parallel plate configuration (25 mm diameter). The edge of the sample was coated with food-grade petroleum jelly. Before starting the measurement, the dough was allowed to rest for 45 min. A constant shear rate of 0.9644/sec was applied to the sample and the viscosity was plotted against time. This moderate shear rate was selected for the viscometry to allow comparison with the shear rates experienced during mixing (≈10/sec) (Bloksma 1990a) while still yielding a sufficient number of data points before edge fracture of the sample occurs. Higher shear rates result in rapid fracture of sample. Dough exhibits a shear thinning response under viscometry; lower viscosities are measured when higher shear rates are used (Phan-Thien et al 1997). Slippage was prevented by using sandpaper glued to the parallel plates before testing (Safari-Ardi and Phan-Thien 1998).

![Fig. 1. Typical uniaxial elongation sequence of bread dough at a strain rate of 0.01/sec. Respective strains and % elongation (deformed length/initial length) for each image. A, 0.46 (158%); B, 2.30 (1,000%); C, 2.89 (1,800%); D, dough rupture. Dough samples broke or ruptured between the plates at strains of ≈2.2 to 4.5.](image)

TABLE I

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glu-A1</th>
<th>Glu-B1</th>
<th>Glu-D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banks</td>
<td>2*</td>
<td>7*+8</td>
<td>2+12</td>
</tr>
<tr>
<td>Hartog</td>
<td>1</td>
<td>7+8</td>
<td>5+10</td>
</tr>
<tr>
<td>Rosella</td>
<td>2*</td>
<td>7+8</td>
<td>2+12</td>
</tr>
<tr>
<td>Sunbri</td>
<td>1</td>
<td>7+8</td>
<td>2+12</td>
</tr>
</tbody>
</table>

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Elongational viscosity curves for the various protein levels differed in viscosity and rupture strain (Fig. 2B–D). For each cultivar, the properties of the dough, as measured by the elongational rupture testing.

**Elongational Testing**

The elongational viscosity remained constant at low strains or extension levels (Fig. 2A). At strains above unity, which corresponded to elongating the sample to ~270% of its original length, the elongational viscosity started to increase rapidly. This sharp increase in elongational viscosity with increasing strain levels is known as strain hardening. A maximum viscosity value was reached during this strain-hardening stage, at which point the dough sample ruptured between the plates. The strain (elongation) and viscosity at which the dough sample broke or ruptured (elongational rupture viscosity and rupture strain) were useful simple measures of dough strain-hardening properties and correspond to extensibility and maximum resistance to extension ($R_{\text{max}}$) measured in traditional dough testing.

Increasing protein content increased the strain-hardening properties of the dough, as measured by the elongational rupture viscosity and rupture strain (Fig. 2B–D). For each cultivar, the elongational viscosity curves for the various protein levels differed only at the point of rupture (Fig. 2B). Elongational rupture viscosity and rupture strain increased with increasing protein levels, a trend consistent with all four cultivars (Fig. 2C and D).

Glutenin-to-gliadin ratio had a more complex effect on dough strain-hardening properties under uniaxial elongation (Fig. 3A). Increases in glutenin-to-gliadin ratio increased the strain-hardening properties as indicated by increasing elongational rupture viscosity. Nevertheless, the greatest effect was observed with the addition of gliadin, which resulted in a considerably decreased elongational rupture viscosity. Unlike the case where protein content was altered, increases in the glutenin-to-gliadin ratio led to increases in elongational rupture viscosity and decreases in rupture strain (Fig. 3B and C). The cultivar differences for rupture strain coincided with HMW-GS constitution with Banks, Rosella and Sunbri (all HMW-GS 2+12) forming one group while Hartog (HMW-GS 5+10) was separate.

**Viscosity Testing**

During viscometry, dough never reached a steady state. Instead, the viscosity increased with shearing, reaching a maximum at which the sample fractured (Fig. 4A and C). Hence, the maximum viscosity was used to compare the different treatments. Increasing the protein content of doughs lowered the measured shear viscosity (shear viscosity curves) and maximum viscosity for all the cultivars (Fig. 4A and B). Increasing the glutenin-to-gliadin ratio had the opposite effect, increasing the shear viscosity and the maximum viscosity of all the cultivars (Fig. 4C and D). Rosella had the lowest viscosity and Hartog the highest.

**Correlation of Parameters**

The results obtained by Uthayakumaran et al (1999) using a small-scale empirical extensigraph-like device showed many significant correlations with the current basic rheological results (Table II). The extensibility measured by the small-scale extension tester (Ext) and the uniaxial elongational rupture strain were highly correlated with $r = 0.924$ when protein content was varied and $r = 0.903$ when glutenin-to-gliadin ratio was varied (Fig. 5A). The maximum resistance to extension and the elongational rupture viscosity had comparably high correlations ($r = 0.757$ and 0.719, respectively) in the two experiments (Fig. 5B). When the data were divided according to high molecular weight glutenin subunit type, the correlations were much stronger ($r = 0.922$ for 2+12 and $r = 0.819$ for 5+10) (Fig. 5B). Both maximum resistance to extension and elongational rupture viscosity had very strong correlations with maximum shear viscosity and negative correlations with Ext and elongational rupture strain. Both correlations were stronger when the glutenin-to-gliadin ratio was modified than when protein content was altered (Table II). The mixograph resistance breakdown showed a strong positive correlation to Ext and elongational rupture strain in the glutenin-to-gliadin ratio experiment and almost as strong a negative correlation in the protein content experiment. Loaf height was positively correlated with elongational rupture strain and Ext and negatively correlated with maximum shear viscosity when the protein content was varied, but these correlations were not significant when glutenin-to-gliadin ratio was varied.

**DISCUSSION**

The use of basic rheological techniques provides a greater level of information on the elongation and shear properties of bread doughs than conventional, empirical techniques have allowed. The results obtained from small-scale empirical extension testing (extensibility and maximum resistance to extension) and basic rheological extension testing (elongational rupture viscosity and strain) were strongly correlated, showing that they measured very similar parameters. There was, however, a certain amount of scatter around the regression line, attributable to variation within the samples as well as to differences in accuracy between the two instruments. Nevertheless, this comparison confirms the validity of these basic rheological measurements.
Elongational testing of a disk of dough gripped at both ends and pulled apart at constant strain rate yields fundamental parameters, which may be related to baking performance. Elongation at low strain rate is similar to bread dough fermentation and oven-rise, where the dough surrounding the expanding gas bubbles is extended along the two axes of the bubble surface, while the bubble wall becomes progressively thinner. Strain hardening, the rapid increase in viscosity at higher strain levels, is thought to be responsible for the ability of dough to expand and retain the gas evolved during fermentation and baking. Studies have shown that flours with good baking quality tend to have much greater strain-hardening behavior than flours that perform poorly in baking (van Vliet et al 1992, 1993). The work of van Vliet et al (1992) utilized compression to achieve biaxial flow, which does not provide dough rupture information. Rupture information is likely to be of particular importance in bread fermentation and baking. Tensile elongation techniques such as the method used in this work, modified alveograph (Dobraszczyk and Roberts 1994, Dobraszczyk 1997), and sheet deformation tests (Morgenstern et al 1996) rupture the dough sample and so provide this information. The latter two techniques involve biaxial flow similar to that experienced by the dough around the expanding gas bubbles. The technique used in this work such as the extensigraph involves uniaxial flow. de Bruijne et al (1990), who also used a uniaxial elongation method, have estimated that uniaxial flow yields viscosities lower than those achieved under biaxial elongation conditions. Janssen et al (1996b) found that the rheological information obtained from biaxial elongation (uniaxial compression and alveograph) and uniaxial elongation (extensigraph) tests on different flour doughs were complementary.

The increasing elongational rupture viscosity and rupture strain observed with increasing protein content due to improved strain-hardening behavior aligns with centuries of bakery experience and cereal science research (Finney and Barmore 1948) that have revealed that bread flours of higher protein levels possess improved baking performance. The presence of greater quantities of protein in the dough serves as a greater reservoir from which the three-dimensional protein structure can develop and impart the gas retaining properties of the dough necessary for good baking quality.

The very different rheological properties of gliadin and glutenin were soon realized after methods for their extraction were developed. Gliadin behaves as a viscous liquid while glutenin behaves more like an elastic solid, leading to the conclusions that gliadin contributes extensibility to the dough, allowing it to flow during fermentation and baking, while glutenin provides elasticity and strength, preventing over-inflation and collapse of the dough (MacRitchie et al 1990, MacRitchie 1992, Khatkar and Schofield 1997).

Increasing the glutenin-to-gliadin ratio increased the elongational rupture viscosity but decreased the rupture strain. Thus, when gliadin predominates, the dough can be elongated further before rupturing, but the rupture viscosity is much lower than when greater proportions of glutenins are present. These effects confirm prior empirical observations of gliadins contributing to flow and extensibility of dough while the glutenins impart the strength or elastic properties to dough (Khatkar and Schofield 1997, Uthayakumaran et al 1999).

The increase in elongational rupture viscosity and rupture strain with increasing protein content confirms the results obtained by Uthayakumaran et al (1999) using a small-scale extension tester. The decrease in elongational rupture strain and increase in elongational rupture viscosity with increasing proportion of glutenin also confirms that gliadins increased the extensibility of bread doughs (Uthayakumaran et al 1999). Unlike these observations, however, the findings reported here of the effect of protein content and glutenin-to-gliadin ratio on the basic rheological properties of
bread dough under elongational flow allow direct comparison with other basic rheological findings. For example, the increasing elongational viscosity of doughs with high glutenin-to-gliadin ratio (i.e., a greater content of longer polymers) has an equivalent finding in studies of the elongational properties of polyethylene melts with both flexible linear molecular structures and branched structures with entanglements (Münstedt and Laun 1981), where the greater the molecular weight, the higher the elongational viscosity.

Viscometry, or simple shear, was used to study the shear flow behavior of the doughs. Because dough does not contain a separate mobile component but is instead composed of a three-dimensional, entangled network, it does not reach a steady state. As the three-dimensional dough network is sheared it presents an increasing resistance to shearing, resulting in an increasing shear viscosity until the network is sheared beyond its physical limit. Further shearing breaks the network structure causing the shear viscosity to decrease. This picture appears to be consistent with network models of bread dough (Bloksma and Bushuk 1988, Bloksma 1990b) and with microscopical studies of hydrated protein (Bernardin and Kasarda 1973).

Increasing dough protein content decreased the maximum shear viscosity while increasing glutenin-to-gliadin ratio increased it. The shear behavior of the dough samples is more difficult to account for than the elongational behavior. It is known that increasing the concentration of a polymer solution and the polymer size increases the shear viscosity (Vinogradov and Malkin 1980). This increase was observed when the glutenin-to-gliadin ratio was increased. The decrease in maximum shear viscosity with increasing protein concentration (in this case, a protein polymer) is an anomalous behavior in terms of polymer solution rheology.

However, a similar effect has been observed in dynamic viscosity measurements of strong bakers and weak biscuit flours, where viscosity decreased with increasing protein content. The strong bakers flour had a lower viscosity than the weak biscuit flour at high strains (Safari-Ardi and Phan-Thien 1998). The same was observed in dynamic viscosity measurements of four blended flour doughs; the two with the highest protein content showed the lowest viscosity, and the two with the lowest protein content showed the highest viscosity (M. Keentok, unpublished data). The anomalous pattern has been seen in measurements on many different polymeric systems, including polymer blends (Larson 1999) and liquid crystals such as poly benzamide (Vinogradov and Malkin 1980) and poly benzyl L glutamate (Larson 1999). In these systems, viscosity increases to a maximum and then decreases as polymer concentration increases. Bread dough can be classed as a polymer blend. The anomalous pattern is also seen in bimodal dispersions (Goodwin 1975), where viscosity decreases with increasing concentration before it increases again. Goodwin (1975) found that the relative viscosity of the suspension can be written as the product of the relative viscosities of the constituent parts. Consequently, for bimodal suspensions such as bread dough, this can give rise to a quadratic (or even higher order) dependence on concentration, showing a decreasing viscosity with increasing concentration and this is what is observed here. An anomalous pattern is also predicted by the Doi theory for dispersions of rigid rods (Larson 1999).

The interaction between a viscoelastic liquid and its suspended particles is a complex one which is reviewed briefly by Larson (1999). Larson (1999) shows that the filled polymer is often less elastic than the polymer alone and also more subject to shear thinning. This may also be the case for bread dough, offering a partial explanation of the anomalous viscosity decrease with increasing protein concentration. Addition of particles to a viscoelastic liquid also introduces a yield stress, which is not present in the unfilled liquid. As a further complication, the current samples also contained lipids in the normal concentration range of the parent flour. Thus bread dough also has a polymer component in addition to being a viscoelastic material (the proteins) with suspended particles (starch). There does not appear to be any literature on such a complex material.

One factor that may account for the unusual shear behavior of bread dough is the effect of the largest component of the dough, namely the starch. Starch-starch and starch-protein interactions may act in a different way in shear and elongational flow, thus highlighting the need to study both types of flow when attempting to characterize the rheology of bread dough.

In this study, two outliers were observed for the parameters tested. They were Hartog, which had the 5+10 HMW-GS, and Rosella, which was a soft wheat. Hartog had much lower elongation to

![Fig. 4. Shear viscometry of Banks. A, Protein contents 80 (–), 100 (—), 120% ( – – – – – ). C, Glutenin-to-gliadin ratios gliadin 0.86 (– – – – – ), gluten 1.25 (—), glutenin 1.42 (– – – ). B and D, Maximum shear viscosity with different protein contents and glutenin-to-gliadin ratios, respectively. Banks (●), Rosella (△), Sunbri (○), Hartog (●). Error bars show ±1 standard error.](image)

![Fig. 5. Extensibility and maximum resistance to extension (R_max) (x axis data from Uthayakumar et al 1999) and uniaxial elongation rupture strain and rupture viscosity (y axis) in two experiments varying in protein content (●, ○) and varying in glutenin-to-gliadin ratio (▲, △). Filled symbols represent Banks, Rosella, and Sunbri with 2+12 HMW-GS; open symbols represent Hartog with 5+10. Lines show overall correlations through all points. Elongational rupture strain = 0.180 × Ext + 0.605 (r = 0.917). Elongational rupture viscosity = 5.42 × R_max + 0.00460 (r = 0.922) for 2+12 and 2.75 × R_max – 0.208 (r = 0.819) for 5+10.](image)

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rupture when compared with Banks, Rosella, and Sunbri with HMW-GS 2+12 (Uthayakumaran et al 1999). The soft wheat Rosella, which also had the lowest protein content, was always an outlier and stood separate in parameters such as elongational viscosity and maximum shear viscosity.

The correlation between elongational rupture strain and loaf height with greater protein content reflects the greater strain hardening and, therefore, improved gas bubble stabilizing properties of the dough. These findings are in agreement with previous studies that have shown a relationship between greater strain-hardening potential of flours and superior baking performance (van Vliet et al 1992). A similar correlation with Ext measured on the small-scale extension tester shows that both tests are measuring similar properties. The negative correlation of loaf height with maximum shear viscosity as protein content is increased is associated with the previously mentioned anomalous shear behavior of shear viscosity decreasing with increasing protein content. No correlations were seen with loaf height when the glutenin-to-gliadin ratio was varied, which is possibly due to the relationship between these parameters being too complex for simple correlation analysis or the experimental design used here to reveal.

CONCLUSIONS

Uniaxial elongational rheology of doughs revealed that the strain-hardening properties of the dough, as indicated by increases in both the elongational rupture viscosity and rupture strain, increased with increasing protein content. Altering the glutenin-to-gliadin ratio had a more complex effect on the elongational properties of the dough, reflecting the different rheological properties of glutenin and gliadin. Adding glutenin increased the rupture viscosity and lowered the rupture strain of the doughs, while addition of gliadin had the opposite effects. Both findings provide rheological support for the widely accepted interpretation of glutenins contributing the elastic and strength characteristics to the dough, and gliadins, the flow properties. The shear viscometry revealed different relationships from that seen in the elongational tests. Adding glutenin increased maximum shear viscosity while increasing protein content lowered the maximum viscosity. Both elongational and shear rheology can reveal differences in the rheology of doughs in which the total protein content and the composition of the protein differ.

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LITERATURE CITED


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