

Dynamic and Elongation Rheology of Yeasted Bread Doughs

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ABSTRACT

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The rheology of yeasted bread doughs is a little-studied field despite yeast's importance in developing bread structure. A method of thermally inactivating the yeast within mixed bread doughs was developed to overcome the difficulty of yeast fermenting during rheological measurements. Sample stabilization by preshearing of dough samples at a stress amplitude of 1 Pa at 1 Hz for 10 sec improved the reliability of small amplitude oscillatory shear measurements, and resting 20 min within the rheometer was sufficient to produce reliable and consistent observations. Small amplitude oscillatory shear measurements were unable to detect any differences between yeasted and nonyeasted doughs nor any changes in linear viscoelastic properties due to fermentation. However, large

strain uniaxial elongation measurements of yeasted doughs revealed a significant progressive decrease in elongational viscosities with fermentation. Size-exclusion HPLC analysis of yeasted doughs showed an increase in unextractable polymeric dough proteins, which were interpreted as evidence of cross-linking and therefore a potential improvement in dough properties. The apparent contradictions between uniaxial elongation and SE-HPLC studies of fermenting yeasted doughs can be attributed to gas bubbles within the dough interrupting the increasingly cross-linked protein network, resulting in the rheological weakness observed for fermenting yeasted doughs.

The baking of bread is almost as old as civilization, with bread even being referred to as the world's oldest convenience food (Orth and Shellenberger 1988). Yeast is the primary leavening agent in baked goods, generating the carbon dioxide that produces the distinctive finely aerated structure of leavened breads. Although cereal rheology is a recent addition to the field of breadmaking, it is one of the oldest branches of rheological study—the world's first rheological instrument was developed in 1836 to study the rheology of dough (Muller 1975). However while yeast plays a crucial role in breadmaking, almost all empirical and fundamental rheological testing and studies are conducted on doughs formulated without yeast. The standard rheological testing instruments in use today by the milling and baking industries (farinographs, mixographs, alveographs, extensigraphs) test nonyeasted doughs. These instruments were originally developed to overcome the inconsistent results obtained from test baking (Brabender 1965). Furthermore, omitting yeast has the advantage of speeding up and making rheological testing simpler. Yeasted doughs have also been avoided in dough rheology research, often with nonyeasted dough rheological findings being compared with baking data to provide a link between nonyeasted rheology and baking performance. The need for rapid testing and the general success of nonyeasted rheological measurements in predicting flour performance in breadmaking has seen the continued avoidance of yeast in dough rheology. However, such an approach does not provide direct information on the rheological changes occurring within bread doughs during fermentation and the stages between mixing and baking of the dough.

Only a handful of studies into the rheological properties of yeasted bread dough have been made. One approach used involved studying the internal gas pressure of the dough in an attempt to derive rheological changes within the fermenting dough (Matsumoto et al 1971, 1973). More direct measurements have also been conducted using extensigraphs on yeasted bread doughs (Kilborn and Preston

1982; Casutt et al 1984) and cracker doughs (Pizzinato and Hosney 1980; Doescher and Hosney 1985). Fundamental rheological studies of yeasted doughs have been made on wheat flour sourdough (Wehrle and Arendt 1998) and on cracker sponge and dough (Oliver and Brock 1997). All these studies of yeasted doughs have allowed the yeast to remain active during the measurements. Such an approach can be problematic in gathering accurate rheological information, particularly when conducting fundamental rheological measurements where the tests are extremely sensitive and may take a long time. Allowing fermentation to continue during rheological measurements would confound these measurements because it is impossible to ascertain what properties result from prior fermentation, the parameter of interest, or from fermentation during measurement. Consequently, the approach taken here was to prevent fermentation during the rheological studies by freezing and thawing the doughs to inactivate the yeast. While the thermal inactivation technique used will also modify the rheological properties of the yeasted doughs, this would be a consistent change that, unlike continued fermentation, would not confound the rheological data because the changes observed with differing fermentation times would be revealed in the rheological data.

The work presented here demonstrates a thermal yeast inactivation technique, and evaluates its effect on the rheological properties of nonyeasted dough. The impact of sample resting in the rheometer and of preshearing in small amplitude oscillatory shear measurements is also evaluated. Utilizing these sample preparation methods, the rheological properties of fermented yeasted doughs is evaluated, as are changes in the protein composition of the doughs. The aim of this work is to establish methodologies for measuring the rheological properties of yeasted doughs from which more extensive rheological characterization and the development of a constitutive rheological model (Phan-Thien et al 1997) will follow.

MATERIALS AND METHODS

Dough Preparation

A standard commercial Australian bread flour, with the product name of Bakers Extra, milled from a blended of cultivars by Weston Milling, Enfield, Sydney, Australia, was used in this study. The physiochemical details of this flour are representative of typical bread flour (Table I). For example, the mechanical dough development (MDD) baking score of 26 for this flour compares favorably with standard bread flour scores of 25 (Mitchell 1989). A formulation of flour, analytical grade salt, sucrose, water, and yeast solution (Table II) were mixed in a 10-g mixograph to peak dough development. Mixing time to peak dough development (Fig. 1) was determined in triplicate from the mixing curves (Gras et al 1990). Dry ingredients were added to the mixograph mixing bowl and blended with a spatula; mixing commenced immediately after the

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water and yeast solution were added. The 6% (w/v) yeast solution was prepared from distilled water and dried instant yeast (Mauripan instant dry yeast, Mauri Yeast Australia Pty. Limited, Sydney, Australia). All ingredients were equilibrated to the air-conditioned laboratory operating temperature (25°C). To provide a link and comparison with previous and contemporary dough studies, nonyeasted control doughs were prepared without yeast and sucrose in their formulation, while the total water added to the dough was kept constant.

Measuring Yeast Gassing Power

The effectiveness of the yeast inactivation techniques was determined by monitoring the volume increase of the treated doughs when placed in an incubator at 37°C. Treated dough pieces (8 g) were placed into 50-mL measuring cylinders, a graduated Perspex rod, with a 21-mm diameter disk at its base, was rested on the dough sample. As the dough piece fermented, the rod rose and the change in dough volume was measured using the graduations on the rod. Dough pieces were monitored over 3 hr. All tests were conducted in triplicate. Drying out of the dough samples was prevented by the disk in contact with the sample at the base of the graduated rod and by another disk covering the top of the glass cylinder. This second disk had a groove that allowed the graduated rod to rise as the dough piece fermented.

Thermal Inactivation Method

Mixed dough pieces were weighed into 4-g portions and subjected to a variety of freeze-thaw regimes. The dough pieces were immediately immersed into liquid nitrogen. Ambient thawing of the dough pieces occurred in an air-conditioned laboratory (25°C) with the dough pieces protected from drying in a plastic bag. A slower two-stage thawing process was also investigated. This involved transferring the frozen dough samples into 2-mL Eppendorf tubes, which were then reimmersed in liquid nitrogen before immersion in 1L of ethanol prechilled to -18°C. The tubes in the ethanol bath were stored overnight in a -18°C freezer. The ethanol container and its cargo of tubes of dough were allowed to warm up to room temperature (25°C) over 5 hr.

TABLE I
Physicochemical Properties of Bakers Extra Bread Flour

Property	Amount
Protein ^a	11.9%
Moisture	13.3%
Optimal water addition ^b	63.0%
Optimal mixing time ^c	360 sec
MDD work input ^d	19.4 Wh/kg
MDD water addition ^d	60.5%
Extensigraph extensibility	19.7 cm
Extensigraph height	505 BU
Bake score ^e	26.0

^a Total protein content by Kjeldahl analysis (AACC 2000).

^b Analysis performed by Weston Milling, Enfield, Sydney, Australia.

^c For 10-g mixograph (Gras et al 1990).

^d Mechanical dough development (MDD) work input and optimum water absorption for 125-g MDD mixers of the New Zealand Institute for Crop & Food, Lincoln, New Zealand (Mitchell 1989; Larsen and Greenwood 1991).

^e Bake score of loaves prepared from dough mixed on 125-g MDD mixers at optimum water absorption and work input levels. Score incorporates volume, weight, and texture of final bake loaf (Swallow and Baruch 1986).

TABLE II
Yeast Dough Composition (dry flour wt basis)

Ingredient	%
Flour	100
Water	73
Salt	2.3
Sucrose	0.9
Yeast	1.4

Rheological Measurements

Small amplitude oscillatory shear measurements were conducted on a controlled stress rheometer (Stresstech, Reologica Instruments AB, Lund, Sweden) using parallel plate configuration of diameter 25 mm to which sandpaper (100 cv) was glued, preventing sample slip. Recent research has revealed the potential for slipping of dough samples during shear measurements and the ability of sandpaper and sample gluing to inhibit slip (Lindborg et al 1997; Safari-Ardi and Phan-Thien 1998). Sample (3 g) was weighed onto the rheometer and compressed between parallel plates at a gap of 2 mm, excess dough was trimmed from the sample. Stress sweep tests were conducted at 1 Hz. The edges of the trimmed dough sample were coated with food-grade petroleum jelly to prevent drying during resting and measurement. Sample temperature was held at 25°C (ambient) by a controlled water bath circulating through the rheometer. All tests were performed in triplicate.

The stress growth in elongational flow was measured under uniaxial tension on a universal testing machine (model SSTM 5000, United Calibration Corp., Huntington Beach, CA) fitted with a 22 N load cell. Dough samples (3.5 g) were compressed to a thickness of 5 mm between two 20-mm diameter plates that had been freshly coated with cyanoacetate glue to ensure adhesion during elongation. Excess dough was trimmed, and the edges coated with petroleum jelly to prevent drying. Dough elongation is achieved by driving the top plate upward at an exponentially increasing speed to attain a

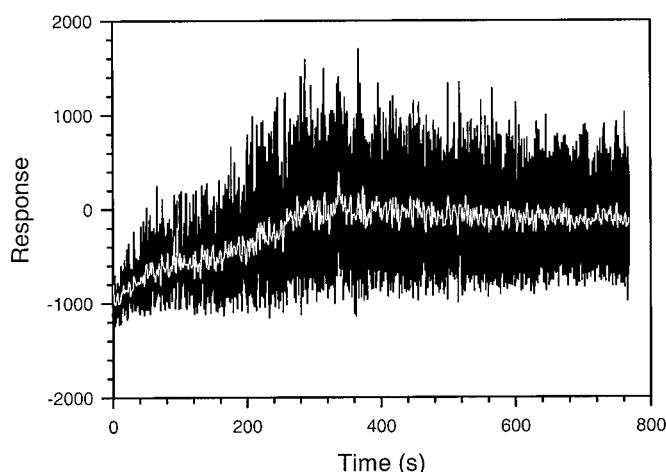


Fig. 1. Mixograph curve of Bakers Extra flour dough used in this study, with peak dough development attained after 360 sec of mixing.

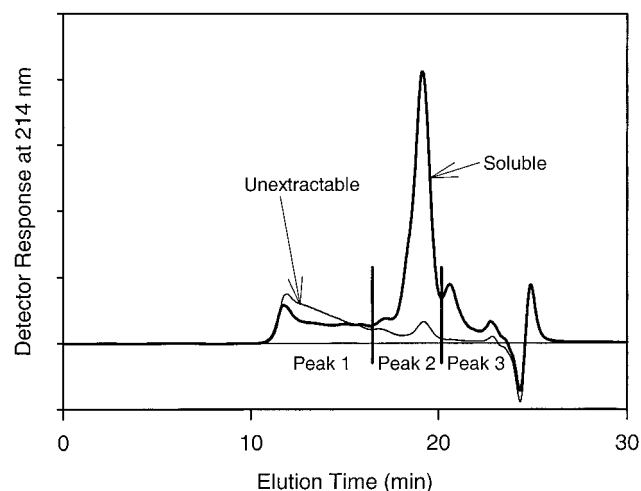


Fig. 2. Size-exclusion HPLC of nonyeasted bread dough showing profiles for SDS soluble (without sonication) protein and unextractable polymeric protein (insoluble in SDS) extracts. Areas under the chromatogram corresponding to the peaks are also shown.

constant strain rate of 0.1/sec. Crosshead movement and data acquisition were controlled by a computer program (QuickBasic, v. 1.1, Microsoft, Seattle, WA). Strain, defined here as Hencky strain, was calculated using $\epsilon = \ln(L/L_0)$, where L_0 is the original length of the sample, i.e., the initial plate separation (5 mm). The elongational viscosity (η^+_{ϵ}) is calculated as the instantaneous axial stress to rate of elongation. The stress, in turn, was calculated at the minimum cross-sectional area of the extending dough sample, which is determined by assuming that the sample volume is constant throughout elongation and that it takes the shape of a cylinder. This assumption is valid at strains >1 (Uthayakumaran et al 2000), at which the dough is subjected to elongational flow, whereas at strains <1 , the flow is a combination of shear and elongation. Tests were conducted in triplicate at 25°C in an air-conditioned laboratory.

Effect of Resting Times and Sample Stabilization

After preparation of the dough samples, they are loaded onto a rheometer and then rested before beginning rheological testing. The effect of this resting period on the rheological properties of non-yeasted doughs under stress sweep and uniaxial elongation were studied. Resting periods investigated included no resting period, 10, 20, 30, and 45 min of resting. The effect of sample stabilization on dough rested for 20 min and subjected to 10 sec of shear at 1, 10, and 100 Pa at 1 Hz was also studied.

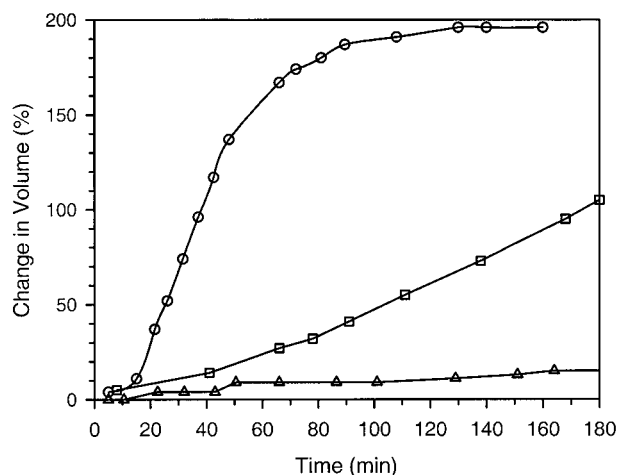


Fig. 3. Effect of thermal treatment on the ability of yeast doughs to ferment when held in a moist environment at 37°C. Comparison of two different treatments are made against a control dough (○). Both treatments involve rapid emersion freezing of the dough pieces in liquid nitrogen followed by thawing in ambient air (25°C) (□), and slow two-stage thawing (△).

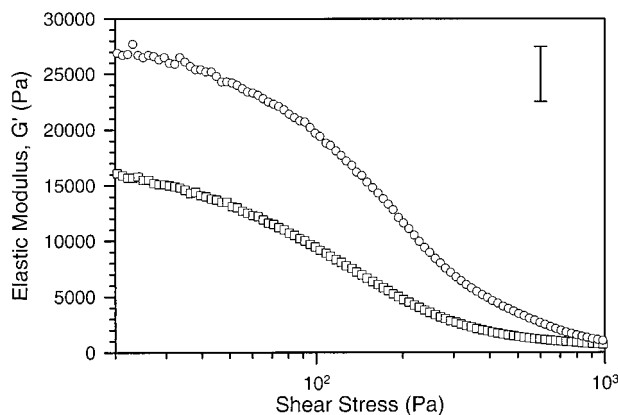


Fig. 4. Effect of freezing and two-stage thawing on the elastic modulus (G') of non-yeasted Bakers Extra flour dough (□) compared to fresh, nonfrozen dough (○). Doughs rested for 20 min, G' measurements were conducted at 1 Hz. Error bar represents pooled standard error of the mean (0.05).

Effect of Fermentation on Dough Rheology

The effect of fermentation on dough properties was investigated by stress sweep and uniaxial elongation of doughs fermented for varying periods of time. Mixed yeasted doughs (16 g) were divided into 4-g pieces and placed inside lidded plastic containers and fermented in an incubator at 37°C. A small vessel holding distilled water was placed with the dough inside the containers to maintain a high humidity and prevent drying out of the fermenting dough pieces. The doughs were fermented for 0, 10, 20, 30, 45, 60, 90, and 120 min before immersion in liquid nitrogen to halt fermentation and subsequent two-stage thawing to inactivate the yeast. To determine the effect of resting that occurs during fermentation, non-yeasted control doughs were prepared and subjected to identical preparation, thermal inactivation, “fermentation”, and testing procedures as the yeasted doughs.

Size-Exclusion HPLC

The size distribution of the polymeric gluten proteins of both the yeasted and non-yeasted doughs subjected to small amplitude oscillatory shear and η^+_{ϵ} measurement were assessed using SE-HPLC as described by Larroque and Bekes (2000) and Gupta et al (1993). Duplicate samples of both the yeasted and non-yeasted doughs were subjected to the freeze-thaw inactivation regime before protein extraction and HPLC analysis. A Beckman system Gold HPLC operating with two 126 Pumps, a 166 Detector (operating at 214 nm) and a 507E Autosampler were used to assess the dough protein extracts. The dough polymeric proteins were extracted using a 0.5% SDS and phosphate buffer, according to the procedure of Gupta et al (1993), which yielded two protein fractions: a SDS-soluble protein (or soluble protein) fraction and an SDS-insoluble polymeric protein fraction. The latter fraction, referred to as unextractable protein, was sonicated to completely disperse the sample. Following filtration through 0.45- μ m PVDF filters, the supernatants were subjected to HPLC analysis at the standard running time of 35 min (flow rate 0.5 mL/min). Integration of chromatograms was performed using Beckman Gold Nouveau software (v. 1.5). The unextractable polymeric protein (UPP) was defined as the ratio of the absolute areas of first peak of the unextractable protein fraction to the sum of the first peaks of both the soluble and unextractable protein extracts. A typical SE-HPLC profile of a non-yeasted dough is given in Fig. 2.

RESULTS AND DISCUSSION

Freezing

A variety of freezing and thawing processes were trailed to obtain the best means of inactivating yeast within the dough before conducting rheological studies on yeast bread doughs. After thermal treatment, dough pieces were incubated at 37°C and the change in volume was measured. Negligible volume change after 2 hr was evidence of successful yeast inactivation. While rapid freezing in liquid nitrogen and thawing at ambient temperatures resulted in a much slower rate of volume increase, there was still a significant level of yeast activity within the first hour (Fig. 3). Use of a two-stage thawing process after liquid nitrogen freezing, first thawing to -18°C and then to ambient (25°C), reduced yeasted dough gas production to negligible levels. The thermal inactivation procedure required that the yeast had begun to ferment before applying the freeze-thaw procedure because yeasted doughs fermented for <10 min exhibit slightly higher levels of residue yeast activity and so were not studied.

The success of the rapid-freeze and two-stage thawing process mirrors earlier research on the lethality of freezing and thawing on yeast suspensions, which showed that slow gradual thawing after rapid freezing minimized yeast survival (Mazur and Schmidt 1968). These same researchers attributed the greater lethality of slower thawing to disruption of internal yeast cell structure by recrystallization and growth of intracellular ice formed during freezing. Furthermore, the ability of yeast cell membranes to stop the passage of ice crystals decreases at -15 to -10°C (Mazur 1970), making slow

thawing at these temperatures more effective at inactivating yeasted doughs. Thus, direct rheological investigation of the effects of fermentation was conducted on yeasted fermented doughs and nonyeasted control doughs that had been frozen in liquid nitrogen and then subjected to two-stage thawing.

Freezing alters the physical properties of wheat doughs. Dynamic elastic modulus (G') of freeze-thaw treated doughs was lower than fresh nonyeasted doughs, with G' being approximately half that of fresh doughs (Fig. 4). Similar changes due to freezing have also been noted in other oscillatory shear studies, with Autio and Sinda (1992) observing a decrease in G' , and Kenny et al (1999) noting a decrease in the complex modulus (G'') of frozen doughs. Likewise freezing and thawing altered large strain uniaxial elongation measurements, lowering the elongational viscosities of freeze-thawed doughs compared with fresh doughs (Fig. 5). These observations correspond with similar decreases in the extensigraph properties of frozen doughs (Inoue and Bushuk 1992; Kenny et al 1999). These freeze-thaw induced changes are thought to result from physical interruption of the dough gluten matrix by ice crystals (Berglund et al 1990, 1991). While freezing affects dough rheological properties, relative changes in the rheological properties of yeasted doughs due to fermentation will still be revealed.

Resting and Sample Stabilization

While the empirical rheological tests that predominate the baking and cereal industries do not yield results in fundamental rheological terms, these tests are conducted under more rigidly defined conditions than the fundamental rheological tests that have been applied to the study of dough rheology. The complex nature of dough rheological behavior makes it very likely that initial conditions influence measured rheological properties. An initial study of the effect of resting time conducted on nonyeasted doughs showed that 20 min of resting yielded reproducible results; however, shorter resting periods were not stable. Although the resting study was determined on fresh nonfrozen doughs, comparison of the sample variation between frozen and fresh doughs rested for 20 min revealed identical levels of variation. Hence, for all experiments, doughs were rested in the rheometers for 20 min before measuring.

Sample stabilization, or preshearing, involves the application of very low strains or stresses within the linear viscoelastic region of the material, to help settle the material down, reducing measurement variation without altering the measured properties. Studies of biological tissues and organs often utilize sample stabilization (Liu and Bilston 1998; Nasser et al 2002). Sample stabilization was applied in one study of wheat gluten rheological behavior (Noel and Brownsey 1990). To determine the best sample stabilization regime for conducting yeasted and nonyeasted dough rheological studies, a range of preshearing stress amplitudes were tested. A preshearing stress amplitude of 1 Pa reduced the standard deviation of G' from 38% down to 7% (Fig. 6). A similar improvement in sample variation was obtained with a stress amplitude of 10 Pa, whereas higher stress amplitudes of 100 and 500 Pa had a detrimental affect on sample variation. Stress amplitudes of 1 and 10 Pa impose respective strain amplitudes of 6×10^{-5} and 3×10^{-4} , well below the strain limit at which dough linear viscoelastic behavior ceases (10^{-3}). Whereas the higher stress amplitudes of 100 and 500 Pa had corresponding strain amplitudes beyond the linear viscoelastic limit, being, respectively 3.5×10^{-3} and 0.13. Thus, a sample stabilization regime of 1 Pa for 10 sec at a frequency of 1 Hz was used for all subsequent shear measurements because at this stress amplitude the deformations involved were well within dough's linear viscoelastic region.

Fermentation and Rheological Dough Properties

No significant differences in the linear viscoelastic elastic (G') and viscous (G'') moduli were observed between the yeasted and nonyeasted doughs during 120 min of fermentation nor did these properties change during fermentation (Table III). Other oscillatory studies of yeasted dough systems have observed decreases in G'

and G'' of cracker sponges (Oliver and Brock 1997) and decreases in the complex viscosity (η^*) of sourdough sponges (Wehrle and Arendt 1998) as fermentation progresses. The inactivation of yeast in the study reported here may account for the constant values of G' and G'' throughout fermentation, whereas the earlier cracker and sourdough studies did not inactivate the yeast before measuring. While the influence of the freeze-thaw process on very low strain behavior of fermented doughs cannot be totally discounted, the influence of dough density on linear viscoelastic properties is likely to have been significant in previous studies. Indeed, the sourdough study noted the dependency of phase angle on CO_2 production rate (Wehrle and Arendt 1998). The yeasted dough samples reported here were thermally inactivated, and this inactivation process and the rheometer loading procedures effectively degassed the samples. This degassing essentially made the densities of yeasted doughs at measurement constant throughout fermentation and also yielded densities only 5% lower than those of nonyeasted doughs (Table III), potentially accounting for the constant linear viscoelastic parameters.

Previous studies have highlighted the inability of small amplitude oscillatory shear measurements to differentiate between nonyeasted doughs made from functionally very different flours (Safari-Ardi and Phan-Thien 1998). While these measurements, conducted by necessity at these very low strain levels, do not reveal changes in inactivated fermenting doughs, the data gathered is an important component in constitutive rheological modeling of yeasted doughs. To identify changes in rheological properties of doughs due to fermentation stress growth experiments are required.

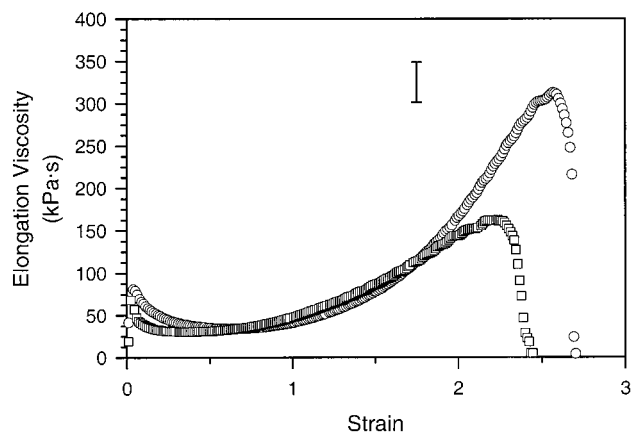


Fig. 5. Effect of freezing and thawing on the extensional viscosity (η^+_{E}) of nonyeasted Bakers dough (\square) with fresh, nonfrozen Bakers dough (\circ) shown for comparison. Doughs rested for 20 min. Error bar represents pooled standard error of the mean (0.05).

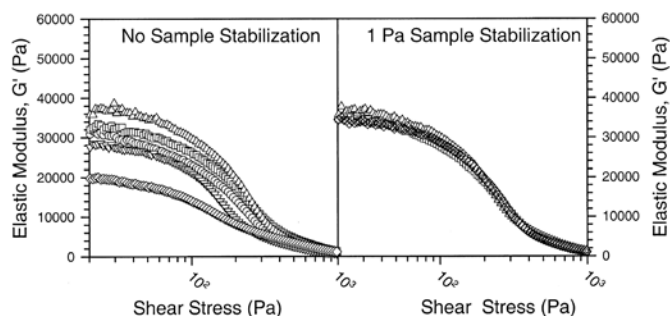


Fig. 6. Effect of sample stabilization using preshearing at a stress amplitude of 1 Pa for 10 sec at 1 Hz on the variability of the elastic modulus (G') of nonyeasted dough. At a shear stress of 30 Pa (within the linear viscoelastic region) G' measurements have a standard deviation of 9,870 Pa (38%) when no sample stabilization is used, which reduces to 2,260 Pa (7%) when it is utilized.

In contrast to the G' and G'' values, η^+_{E} showed clear and significant changes in freeze-thaw treated yeasted doughs as they were fermented, with η^+_{E} at rupture decreasing with increasing periods of fermentation (Fig. 7). This response was not observed with frozen-thawed nonyeasted doughs, whose η^+_{E} at rupture did not change over the 120-min fermentation period. Decreasing yeasted dough η^+_{E} at rupture indicates that fermentation weakens the strain hardening properties of yeasted doughs. Similar weakening of yeasted dough elongation properties due to fermentation has been noted in extensigraph studies of active bread doughs (Kilborn and Preston 1982; Casutt et al 1984) and cracker sponges (Pizzinato and Hosney 1980; Doescher and Hosney 1985).

Size Exclusion HPLC

As with the uniaxial elongation observations, SE-HPLC analysis revealed significant changes in polymeric protein composition of yeasted doughs during fermentation, whereas nonyeasted doughs remained unchanged (Fig. 8). Yeasted doughs started out with a proportion of UPP approximately half that of nonyeasted dough, which increased during fermentation to a level close to that of nonyeasted dough. Changes in the UPP of yeasted doughs resulted from a decrease in soluble glutenin proteins (Peak I of soluble fraction, Table IV) and an increase in insoluble glutenin proteins (Peak I of unextractable fraction, Table IV). These findings concur with other studies of yeasted doughs. With Borneo and Khan (1999) finding a decrease in the soluble glutenin proteins during breadmaking and fermentation, and Veraverbeke et al (1999) noting an increase in unextractable protein throughout fermentation.

Changes in the size distribution of polymeric protein during fermentation observed in this study were interpreted as resulting from cross-linking of dough proteins either directly due to fermentation or to a secondary process associated with it. From a quality point of view, the presence of greater quantities of large polymeric flour proteins is positively correlated with baking quality and other empirical measures of dough strength (Dachkevitch and Autran 1989; Singh et al 1990; Gupta et al 1993; Weegels et al 1996). The shift in polymeric protein size distribution to larger polymeric proteins would be expected to correspond to improved dough strength. But declining η^+_{E} of yeasted doughs during fermentation contradicts this prediction. A likely source of this weakening action is the increasing gas volume of fermenting dough. The presence of expanding gas bubbles within yeasted doughs would interrupt the increasingly cross-linked protein network, counteracting any improvement in

rheological properties afforded by these changes in polymeric protein size distribution. The large differences between the UPP of yeasted and nonyeasted doughs at the early stages of fermentation suggest that yeast influences polymeric dough protein composition during mixing. The mechanism of this action is unknown, although yeast enzymes may play a role.

There are, however, some anomalies relating to the rheological and dough polymeric protein data. At the early stages of fermentation, before significant gas bubble growth has occurred in the yeasted doughs, the yeasted and nonyeasted doughs exhibit very similar elongational behavior (Fig. 7) when, however, they possess quite differing UPP levels (Fig. 8). Explanations of this aberrant behavior are not known. However, such findings suggest that the relationship between dough polymeric proteins and elongational behavior is more complex than previously supposed.

CONCLUSIONS

Rapid freezing followed by two-stage thawing of yeasted dough halts yeast activity within the dough, allowing rheological studies to be conducted without the confounding influence of fermentation occurring during measurement. The freeze-thaw process weakened G' and η^+_{E} of nonyeasted doughs. Studies of rheometer testing con-

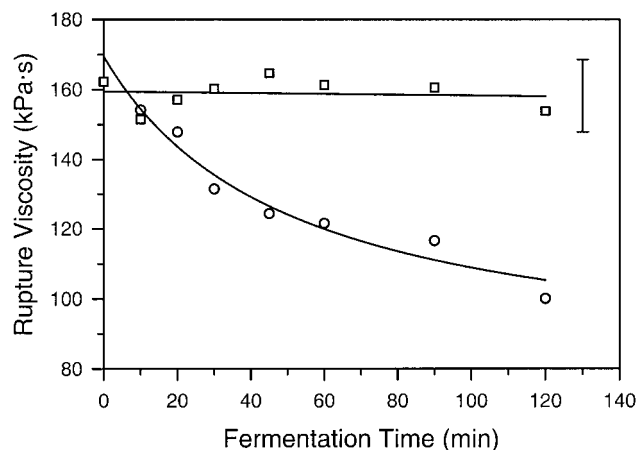


Fig. 7. Elongational viscosity (η^+_{E}) at rupture of yeasted (\circ) and nonyeasted (\square) doughs subjected to freeze-thaw inactivation after being fermented for varying periods of time. Points are mean values of triplicate measurements. Error bar represents pooled standard error of the difference (0.05).

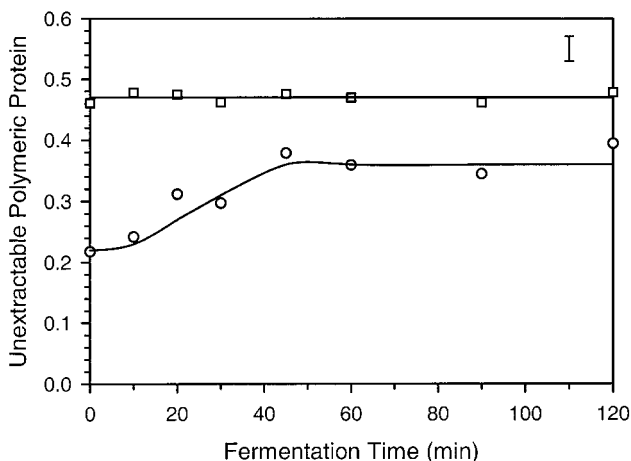


Fig. 8. Changes in unextractable polymeric protein (UPP) of yeasted (\circ) and nonyeasted (\square) doughs during fermentation as assessed by SE-HPLC. UPP corresponds to the ratio of the unextractable protein fraction's first peak to the sum of the first peaks from both the soluble and unextractable protein fractions. Mean values of duplicate measurements are displayed; error bar represents pooled standard error of the mean (0.05).

TABLE III

Effect of Fermentation on Dough Density and Linear Viscoelasticity

	G'	G''	Dough Density (kg/m^3) ^a
Nonyeasted	16,500	5,400	1.24
Yeasted	16,300	5,200	1.18
SEM ^b	2,100	700	0.03

^a Dough densities of doughs after thermal inactivation and associated degassing.

^b Pooled standard error of the mean at $P < 0.05$.

TABLE IV

Size-Exclusion HPLC Analysis of Yeasted Doughs

Time (min)	Soluble Fraction Area (%)		Insoluble Fraction Area (%)
	Peak I	Peak II	Peak I
0	40.1	48.1	78.7
10	39.2	49.0	77.2
20	38.5	49.7	78.3
30	37.5	50.3	81.8
45	37.4	50.5	83.0
60	38.1	50.0	85.7
90	37.6	50.3	83.8
120	37.1	50.6	83.1
SEM ^a	1.9	1.3	2.1

^a Pooled standard error of the mean at $P < 0.05$.

ditions revealed resting periods of 20 min yielded consistent results and use of a preshearing sample stabilization step reduced sample variability of small amplitude oscillatory shear measurements. Yeasted and nonyeasted doughs had similar G' and G'' , which were not influenced by fermentation, possibly due to degassing of the doughs during freeze-thaw treatment. As has been observed with nonyeasted doughs, distinguishing of rheological differences in doughs is most effective when large strain measurements, like stress growth in elongational flow (η^+_{E}) or in shear viscometry (η^+_{S}) are employed.

Fermentation had a significant effect on η^+_{E} with η^+_{E} at rupture of the yeasted dough decreasing during fermentation while nonyeasted doughs were unaffected. This weakening action of fermentation on yeasted dough η^+_{E} was in contrast to SE-HPLC analysis of dough polymeric protein composition. SE-HPLC analysis revealed an increase in UPP of yeasted doughs during fermentation, which is interpreted as evidence of increased cross-linking of dough proteins with fermentation. As with the uniaxial elongational findings, the UPP of nonyeasted doughs remained unaffected. The presence of expanding gas bubbles would interrupt and offset any strengthening action of the increasingly cross-linked structure on the rheological properties of the fermenting doughs. Fermentation has a more complex influence on dough protein composition and rheological properties than studies of nonyeasted doughs alone can reveal. Therefore, to gain greater understanding of fermentation and breadmaking, more effort needs to be focused on studies of yeasted doughs.

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LITERATURE CITED

- American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Method 46-11A. The Association: St. Paul, MN.
- Autio, K., and Sinda, E. 1992. Frozen doughs: Rheological changes and yeast viability. *Cereal Chem.* 69:409-413.
- Berglund, P. T., Shelton, D. R., and Freeman, T. P. 1990. Comparison of two sample preparation procedures for low-temperature scanning electron microscopy of frozen bread dough. *Cereal Chem.* 67:139-140.
- Berglund, P. T., Shelton, D. R., and Freeman, T. P. 1991. Frozen bread dough ultrastructure as affected by duration of frozen storage and freeze-thaw cycles. *Cereal Chem.* 68:105-107.
- Borneo, R., and Khan, K. 1999. Protein changes during various stages of breadmaking of four spring wheats: Quantification by size-exclusion HPLC. *Cereal Chem.* 76:711-717.
- Brabender, C. W. 1965. Physical dough testing: Past, present and future. *Cereal Sci. Today* 10:291-304.
- Casutt, V., Preston, K. R., and Kilborn, R. H. 1984. Effects of fermentation time, inherent flour strength, and salt level on extensigraph properties of full-formula remix-to-peak processed doughs. *Cereal Chem.* 61:454-459.
- Dachkevitch, T., and Autran, J. C. 1989. Prediction of baking quality of bread wheats in breeding programs by size-exclusion high-performance liquid chromatography. *Cereal Chem.* 66:448-456.
- Doescher, L. C., and Hoseney, R. C. 1985. Saltine crackers: Changes in cracker sponge rheology and modification of a cracker-baking procedure. *Cereal Chem.* 62:158-162.
- Gras, P. W., Hibberd, G. E., and Walker, C. E. 1990. Electronic sensing and interpretation of dough properties using a 35-g mixograph. *Cereal Foods World* 35:568-571.
- Gupta, R. B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. *J. Cereal Sci.* 18:23-41.
- Inoue, Y., and Bushuk, W. 1992. Studies on frozen doughs. II. Flour quality requirements for bread production from frozen dough. *Cereal Chem.* 69:423-428.
- Kenny, S., Wehrle, K., Dennehy, T., and Arendt, E. K. 1999. Correlations between empirical and fundamental rheology measurements and baking performance of frozen bread dough. *Cereal Chem.* 76:421-425.
- Kilborn, R. H., and Preston, K. R. 1982. A modified extensigraph procedure for measuring the stretching properties of fermented dough. *Cereal Chem.* 59:381-384.
- Larroque, O. R., and Bekes, F. 2000. Rapid size-exclusion chromatography analysis of molecular size distribution for wheat endosperm protein. *Cereal Chem.* 77:451-453.
- Larsen, N. G., and Greenwood, D. R. 1991. Water addition and the physical properties of mechanical dough development doughs and breads. *J. Cereal Sci.* 13:195-205.
- Lindborg, K. M., Trägårdh, C., Eliasson, A. C., and Dejmek, P. 1997. Time-resolved shear viscosity of wheat flour doughs—Effect of mixing, shear rate, and resting on the viscosity of doughs of different flours. *Cereal Chem.* 74:49-55.
- Liu, Z., and Bilston, L. 1998. Linear viscoelasticity properties of bovine liver tissue. Pages 153-156 in: Proc. 8th Nat. Conf. on Rheology. Q. D. Nguyen and R. R. Huilgol, eds. The University of Adelaide: Australia.
- Matsumoto, H., Nishiyama, J., and Hlynka, I. 1971. Internal pressure in yeasted dough. *Cereal Chem.* 48:669-676.
- Matsumoto, H., Nishiyama, J., and Hlynka, I. 1973. Internal pressure in yeasted dough. II. *Cereal Chem.* 50:363-371.
- Mazur, P. 1970. Cryobiology: The freezing of biological systems. *Science* 168:939-949.
- Mazur, P., and Schmidt, J. J. 1968. Interactions of cooling velocity, temperature, and warming velocity on the survival of frozen and thawed yeast. *Cryobiology* 5:1-17.
- Mitchell, T. A. 1989. Methods used in monitoring and controlling the quality of bread with particular reference to the mechanical dough development process. Pages 313-331 in: *Modern Methods of Plant Analysis*. Vol. 10. H. F. Linskens and J. F. Jackson, eds. Springer-Verlag: Heidelberg, Germany.
- Muller, H. G. 1975. Rheology and the conventional bread and biscuit-making process. *Cereal Chem.* 52:89r-105r.
- Nasseri, S., Bilston, L. E., and Phan-Thien, N. 2002. Viscoelastic properties of pig kidney in shear, experimental results and modelling. *Rheol. Acta* 41:180-192.
- Noel, T. R., and Brownsey, G. J. 1990. The rheological character of gluten. Pages 145-154 in: *Rheology of Food, Pharmaceutical and Biological Materials with General Rheology*. R. E. Carter, ed. Elsevier Science: Barking, Essex, UK.
- Oliver, G., and Brock, C. J. 1997. A rheological study of mechanical dough development and long fermentation processes for cream-cracker dough production. *J. Sci. Food Agric.* 74:294-300.
- Orth, R. A., and Shellenberger, J. A. 1988. Origin, production and utilization of wheat. Pages 1-14 in: *Wheat: Chemistry and Technology*. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Phan-Thien, N., Safari-Ardi, M., and Morales-Patiño, A. 1997. Oscillatory and simple shear flows of a flour-water dough: A constitutive model. *Rheol. Acta* 36:38-48.
- Pizzinato, A., and Hoseney, R. C. 1980. Rheological changes in cracker sponges during fermentation. *Cereal Chem.* 57:185-188.
- Safari-Ardi, M., and Phan-Thien, N. 1998. Stress relaxation and oscillatory tests to distinguish between doughs prepared from wheat flours of different varietal origin. *Cereal Chem.* 75:80-84.
- Singh, N. K., Donovan, R., and MacRitchie, F. 1990. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of breadmaking quality. *Cereal Chem.* 67:161-170.
- Swallow, W. H., and Baruch, D. W. 1986. Loaf evaluation. Report no. WRI 86/103. Wheat Research Institute, DSIR: Christchurch, NZ.
- Uthayakumaran, S., Newberry, M., Keentok, M., Stoddard, F. L., and Bekes, F. 2000. Basic rheology of bread dough with modified protein content and glutenin-to-gliadin ratios. *Cereal Chem.* 77:744-749.
- Veraverbeke, W. S., Courtin, C. M., Verbruggen, I. M., and Delcour, J. A. 1999. Factors governing levels and composition of the sodium dodecyl sulphate-unextractable glutenin polymers during straight dough breadmaking. *J. Cereal Sci.* 29:129-138.
- Weegels, P. L., Hamer, R. J., and Schofield, J. D. 1996. Critical review: Functional properties of wheat glutenin. *J. Cereal Sci.* 23:1-18.
- Wehrle, K., and Arendt, E. K. 1998. Rheological changes in wheat sourdough during controlled and spontaneous fermentation. *Cereal Chem.* 75:882-886.

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