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MONITORING DOUGH FERMENTATION USING ACOUSTIC WAVES

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Fermentation of the sugars in bread dough produces carbon dioxide which diffuses through the dough matrix into the gas cell nuclei formed during dough mixing. As a result, the void fraction of the dough increases and dough density decreases. It is shown in this paper that low intensity ultrasound can be used to monitor changes in the void fraction of this opaque material, thus providing real-time information on changes in the structure of the dough during fermentation. Doughs were mixed under two different mixer headspace conditions, and the ultrasonic velocity and attenuation measured as a function of fermentation time. The results of the ultrasonic experiments were compared with changes in dough density (measured independently but under the same experimental conditions). As fermentation time increased (and gas cells expanded), the ultrasonic velocity decreased and the attenuation increased. At early fermentation times, a substantial drop in velocity was observed before the density changed appreciably, indicating that yeast activity has two independent effects on dough properties: modifying the elasticity of the dough matrix and expanding the gas cells. Ultrasound therefore has the potential to provide novel information on technological issues of critical importance to the cereals' processing industry.

Keywords: ultrasound; bread dough; fermentation; density; structure.

INTRODUCTION

Measurements of ultrasonic velocity and attenuation have been used to characterize the physical properties of many food systems (Ghaedian *et al.*, 1998; Gunasekaran and Ay, 1996; McClements *et al.*, 1993; Mizrach *et al.*, 1989). Because ultrasonic techniques have the advantage of acquiring measurements rapidly, the measurement of ultrasonic properties can in some cases be performed on-line (Forrest, 1996; Saggin and Coupland, 2001). The ultrasonic velocity and attenuation coefficient can then be related to various physical properties of the foods being tested, or they can be used to control the process conditions of a particular unit operation.

Ultrasonic evaluations of the properties of dough-based products are not as widespread as they are in other sectors of the food and chemical processing industries. Part of the reason appears to be the strong attenuation of ultrasound in dough (Létang *et al.*, 2001; Moorjani, 1984). Therefore, much use is made in breadmaking processes of empirical measurements of the mechanical properties of dough using instruments such as the farinograph, the alveograph and the mixograph (Bloksma and Bushuk, 1988) although large strain (Charalambides *et al.*, 2002; Safari-Ardi and Phan-Thien, 1998) and small strain (Edwards *et al.*, 2002; Safari-Ardi and Phan-Thien, 1998) measurements of fundamental mechanical properties of dough and dough-based products are receiving greater emphasis.

One ingredient that affects the mechanical properties of dough is its gas content (Charalambides *et al.*, 2002). Indeed, it has been stated that the various operations of breadmaking 'may be viewed as a series of aeration stages' (Campbell *et al.*, 1998). Therefore, a technique such as ultrasound, which is very sensitive to the numbers and sizes of gas inclusions within a solid or liquid matrix (Leighton, 1997), appears to have great potential for process monitoring of dough properties, either at-line or on-line. The objective of this paper is to show how low-frequency ultrasound (50 kHz) can be used to study the dynamics of dough fermentation. By monitoring the changes in ultrasonic velocity and attenuation that occur during fermentation, information on the effect of fermentation on both dough matrix properties and the expansion of the gas cells within the dough matrix is obtained.

METHODS AND MATERIALS

Dough samples were prepared from flour milled from Canadian Western Red Spring wheat (CWRS, grade no. 1), with a flour protein content of 12.4%. All wheat was milled in the Canadian International Grains Institute (CIGI) pilot mill (Winnipeg, Manitoba, Canada). Dough was prepared

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using the Canadian Short Process Method (Preston *et al.*, 1982), which was carried out using flour (100 g), salt (2.4 g), yeast (4.2 g) and water (63 ml). This water content gave optimum absorption for the first batch of flour. The dough was mixed at 165 rpm to 10% past peak consistency at 30°C in a GRL-200 mixer. Two mixer headspace conditions were selected: ambient conditions, i.e. at atmospheric pressure, and under vacuum, where the mixer was sealed with vacuum grease and a vacuum pump continuously evacuated the headspace of the mixer to attain a pressure during mixing of 0.13 atm. At the end of mixing, two 4 g samples of dough were immediately removed from the dough piece, one for the ultrasonic measurements and one for the density measurements (first readings obtained approximately 4 min after completion of mixing). This allowed both density and ultrasonic measurements to be performed simultaneously using samples from the same dough batch. The rest of the dough was discarded, so all measurements were obtained from fresh dough samples. At least three doughs were prepared for the experiments.

The density of the fermenting dough was measured as a function of fermentation time using a digital imaging technique in which the dough sample was allowed to ferment in the proving cabinet at normal proving conditions, i.e. 37°C and 83% R.H (Preston *et al.*, 1982). Each sample was weighed accurately and placed between two 3 cm-thick acrylic plates. The dough was squashed slowly to a preset thickness, which could be adjusted by using a set of 1 mm-thick glass slides inserted between the two acrylic plates. To restrict expansion of the dough during fermentation to the radial direction only, the two plates were clamped together. The clamped plates were then placed in the proving cabinet and mounted directly below a digital camera (Figure 1). Digital images of the radial expansion of the fermenting dough were taken every 2 min. To determine the density of the dough as a function of fermentation time, the area of the expanding dough was measured using image analysis software (Scion Image, www.scioncorp.com). Since the distance between the acrylic plates was fixed throughout the experiment and the mass had been measured, the density (ρ) was easily found from: $\rho = m/LA(t)$, where L is the sample thickness (maintained at 1.97 mm) and $A(t)$ is the area of the dough as a function of fermentation time (t). Calibration of the area was achieved by placing the clamped system on a piece of graph paper, and correcting for differences in the distance between the camera and either the dough or the graph paper. By following this procedure, the dough density was measured under exactly the same conditions as those used in the ultrasonic experiments, where the dough had to be placed between the two transducers, restricting dough expansion to the radial direction only.

The ultrasonic velocity was measured using a PUNDIT generator/receiver, with a pair of longitudinal transducers having a nominal operating frequency of 50 kHz (PUNDIT 6, CNS Farnell, Borehamwood, UK). The detected waveforms were signal averaged on a digitizing oscilloscope (Tektronix model TDS 420A, Tektronix Canada Inc., Toronto), and downloaded to a computer for subsequent analysis. To ensure that the boundary conditions on the sample were the same for both the ultrasonic and density measurements, the dough samples were placed between two 3 cm-thick acrylic plates, identical to those used in the density measurements, and adjusting the dough thickness to 1.97 mm (Figure 1). The two transducers were coupled to the outer sides of the acrylic plates using a thin gel layer (Ultragel, Diagnostic Sonar Ltd, Scotland). The velocity was calculated from the time taken for a pulsed signal to travel from one side of the sample to the other. The measurements were done in two steps. In the first step, a reference waveform was acquired for the signal passing through one of the acrylic plates. After the reference signal was acquired, the sample was placed between the two transducers and the sample waveform was acquired as a function of fermentation time. The transit time difference between the two waveforms (reference and sample) was then measured by determining the time shift required to align the pressure oscillations at the leading edge of the pulses.

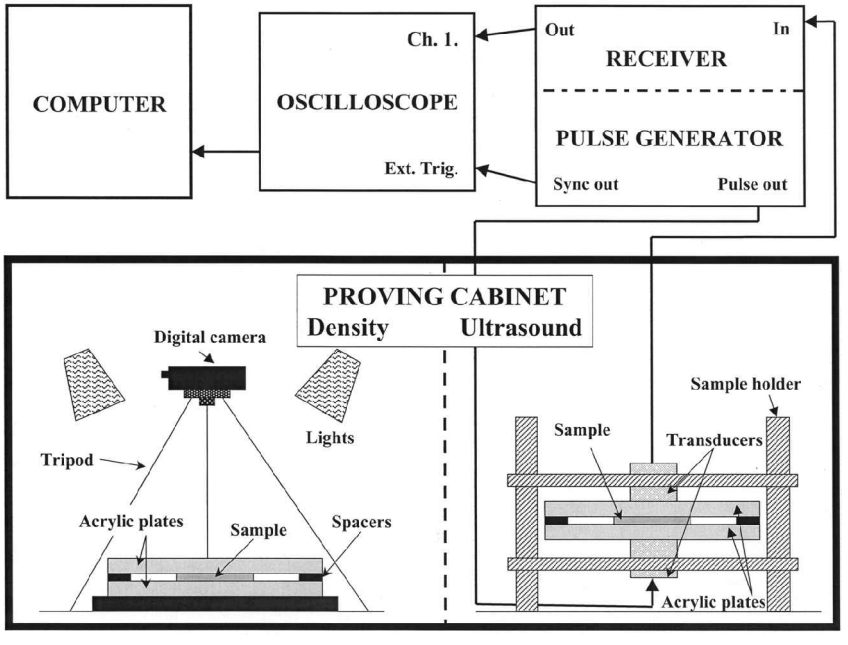

Figure 1. Block diagram of the apparatus used for ultrasonic and density measurements on fermenting dough.

(Measuring the difference between the initial arrival times of the pulses eliminated complications from multiple reflections in the plastic plates.) This time difference was then corrected for the propagation time through the second acrylic plate to obtain the time taken for the signal to travel through the sample, Δt. The transit time in conjunction with the sample thickness L yields the velocity of sound through the sample, $v = L/\Delta t$. The change in attenuation as a function of fermentation time was monitored by measuring the voltage on the oscilloscope corresponding to the peak height of the transmitted ultrasonic pulse.

RESULTS AND ANALYSIS

Density of Fermenting Dough

Typical images of the fermenting dough at various fermentation times are shown in Figure 2. These images show the radial expansion of the dough due to inflation of the air nuclei within the dough as CO_2 is produced by the yeast. Dough density as a function of fermentation time for the doughs mixed at ambient pressure and under vacuum (0.13 atm) is shown in Figure 3. Regardless of mixer headspace pressure, dough density decreases as a function of fermentation time. This result is expected, and is consistent with density measurements made on fermenting doughs in xylene (Campbell *et al.*, 2001), although the density decrease in this study is not as rapid. At the beginning of fermentation, the density of the dough mixed under vacuum is higher than when the dough is mixed at ambient pressure. This initial density difference is caused by the occlusion of fewer bubbles in the dough that has been mixed at reduced pressure. Furthermore, the density changes relatively slowly at early fermentation times, especially in the vacuum-mixed dough. This lag in the density variation relative to the onset of yeast activity results from both the time taken for the yeast to start fermenting the sugars and the time taken for the CO_2 to diffuse through the dough matrix to the air nuclei (Cooper and Reed, 1968). Since at the beginning of fermentation, the vacuum-mixed dough has fewer bubbles per unit volume than the dough mixed at ambient pressure (Campbell *et al.*, 1998, 2001), the diffusion path for CO_2 to reach the air nuclei must be longer. This will cause the vacuum-mixed dough to exhibit a longer lag time than its ambient pressure counterpart. For both dough types, the density drops by about the same amount with time suggesting that at the end of fermentation roughly the same amount of CO_2 is produced and retained in both cases.

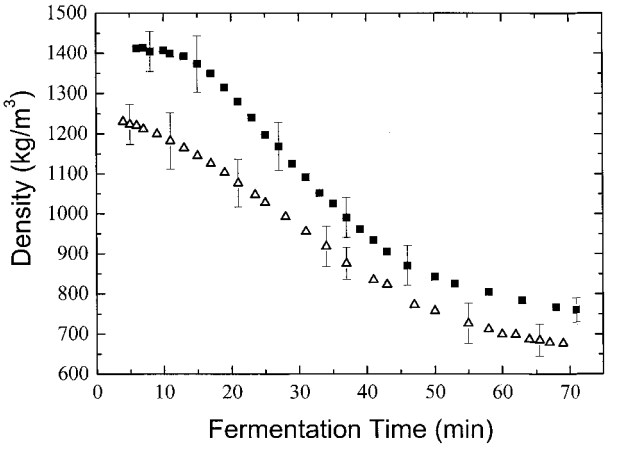

Figure 3. Density of fermenting dough that had been mixed under vacuum (■) and at atmospheric pressure (△).

Ultrasonic Velocity and Attenuation and Dough Properties

For a longitudinal ultrasonic ballistic pulse, which travels coherently through the medium without scattering out of the forward direction, the change in signal amplitude is related to the attenuation coefficient, α, as follows:

$$A(t) = A_0 \exp\left[-\alpha(t)\frac{L}{2}\right] \quad (1)$$

where $A(t)$ is the signal amplitude at time t, A_0 is the signal amplitude at the leading edge of the dough sample, and L is the sample thickness. Here α is the intensity attenuation coefficient, which is twice the amplitude attenuation coeffi-

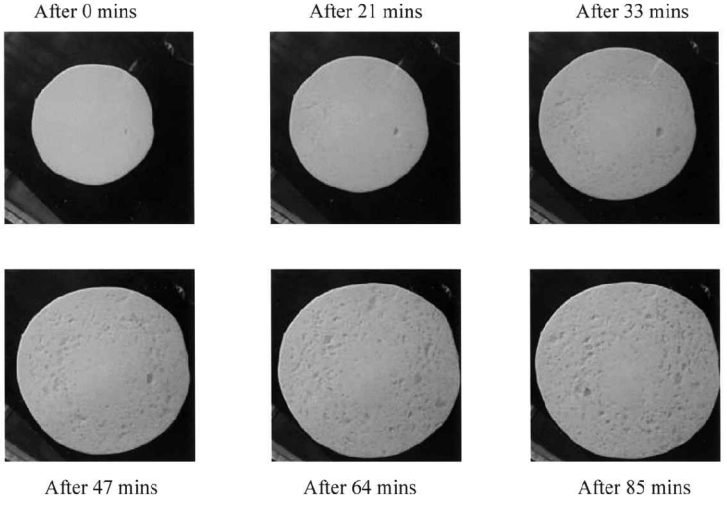

Figure 2. Digital images of fermenting dough at various times. The scale shown is the same for all images.

cient (Edmonds, 1981). For a heterogeneous system such as dough, α is the sum of two loss mechanisms: absorption and scattering. Research has shown that the attenuation coefficient is affected by the air content of the dough (Elmehdi, 2001) and by its water content (Létang *et al.*, 2001). Therefore, since both void fraction and the dough matrix affect the attenuation coefficient, it is expected that attenuation would change during fermentation.

The longitudinal phase velocity of ultrasonic waves is related to the longitudinal modulus, β, of the dough by (Page *et al.*, 1995):

$$v = \sqrt{\frac{\beta}{\rho}} \tag{2}$$

where ρ is dough density. For a solid material exhibiting elastic properties, the modulus $\beta = \kappa^{-1} + \frac{4}{3}\mu$ where κ is the compressibility and μ is the shear modulus. The compressibility and the shear modulus are determined by the intermolecular forces acting within the dough, and by the dough's structure. Therefore, measurements of ultrasonic velocity, and independent measurements of dough density (Figure 3), will permit the mechanical properties of the dough to be evaluated during fermentation.

Ultrasonic Velocity Measurements

The ultrasonic velocity as a function of fermentation time for dough mixed under two different mixer headspace conditions is shown in Figure 4. At the beginning of fermentation, the velocity is substantially greater in the dough mixed under vacuum than its ambient counterpart. This is attributed to the presence of fewer air nuclei in the dough (Baker and Mize, 1941; Campbell *et al.*, 1998) as well as to a large increase in the shear modulus of the dough matrix when dough is mixed at low pressure (Elmehdi, 2001). As the yeast's metabolic activities generate CO_2 in the dough, the velocity drops substantially over the course of the first 20 min for doughs mixed under both conditions. At later fermentation times, the velocities essentially converge, suggesting that, as comparable amounts of CO_2 have been generated and the gas cell volumes are similar, the dough moduli are also similar. While both the velocity and density decrease with fermentation time (Figures 3 and 4), there is not a one-to-one correspondence between the changes in these two parameters, especially for the vacuum-mixed dough at early times where the velocity drops precipitously but the density is almost constant. Therefore, additional effects, other than the increase in void fraction, must cause this initial dramatic drop in velocity.

One possible explanation is that the large drop in velocity is associated with a relaxation of the initial internal pressure within the dough that had been caused by confining the sample between the transducers. To investigate this hypothesis, the density and ultrasonic velocity were measured as a function of fermentation time for samples of various masses (2, 4 or 7 g; doughs mixed only at atmospheric pressure). In these experiments the sample thickness was fixed at 1.97 mm, so that the net effect of changing the mass was to change the amount by which the two transducers stressed the dough sample, and hence altered the internal pressure within the dough. It can be seen from Figure 5 that the variation in both the density and the velocity is similar in magnitude (within experimental error) for the samples of different masses. Hence the change in the velocity and density appear to be independent of the internal pressure in these experiments, and so the rapid drop in the velocity at early fermentation times is not caused by the stress induced by the transducers. It is interesting to note that the magnitude of the velocities in Figures 4 and 5 are different due to a change in the wheat flour used (new crop year). The same water to flour ratio was used because it has been shown that moisture content affects the ultrasonic velocity in dough

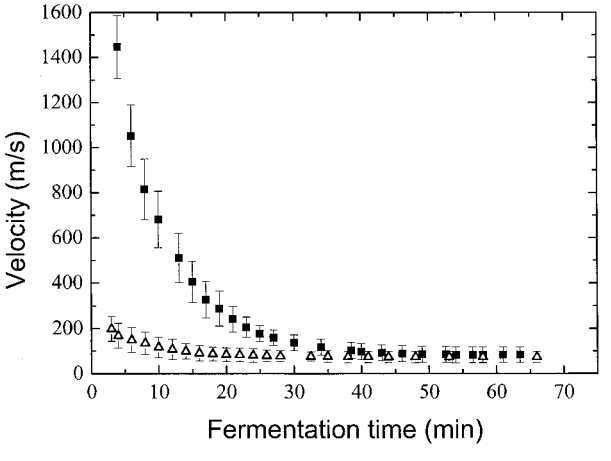

Figure 4. The velocity of ultrasound in fermenting dough that had been mixed under vacuum (■) and at atmospheric pressure (△).

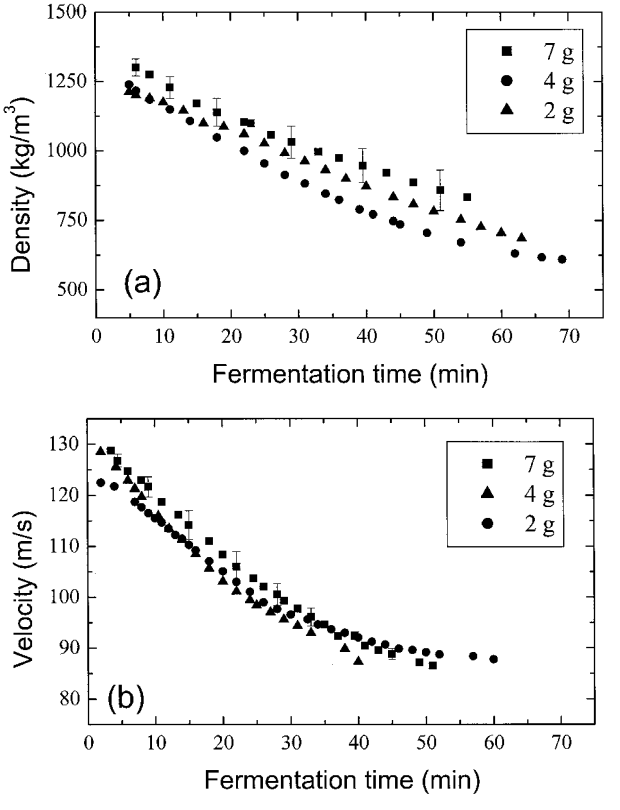

Figure 5. (a) The density of fermenting dough measured using dough of masses 2, 4 and 7 g. The dough was mixed at atmospheric pressure. (b) The velocity measured for the samples in (a).

(Létang *et al.*, 2001). From these results, it is apparent that how the water is bound in the dough structure is also a determinant of the ultrasonic velocity.

A second possible mechanism that could cause the reduction in the velocity is the effect of the drop in the pH value of the dough (Scanlon *et al.*, 2002). The dough, which has substantial buffering capacity, has an initial pH of 5.5 (Eliasson and Larsson, 1993), which drops due to fermentation to a pH as low as 4.5 (Pyler, 1973). The fall in pH arises from the saturation of carbon dioxide in the aqueous phase generating carbonic acid within the dough matrix. Although the number of ionizable side-chains in the amino acids of the gluten proteins is small (Wu and Dimler, 1963), a drop in pH during fermentation will cause the proteins within the dough matrix to acquire a larger number of positive charges. Those regions of the gluten proteins that have a higher concentration of positive charges in close proximity will repulse each other. It is expected that the repulsion within the gluten network structure will increase with increasing positive charge (Eliasson and Larsson, 1993), i.e. with decrease in pH, so that the mechanical compliance of the gel will increase. The increased compliance will be apparent as a sharp reduction in the velocity of the ultrasound at the early stages of fermentation where pH is dropping rapidly (Pyler, 1973).

To test whether the drop in pH, rather than other metabolic products of the yeast, was responsible for the large drop in velocity, doughs were formulated without yeast using hydrochloric acid in place of water. In this way, doughs were prepared with values of pH comparable to those attained with yeast before and after fermentation, but without the complication of large changes in density arising from the expansion of the gas nuclei within the dough by CO_2. From Table 1, it is evident that the velocity dependence on pH is affected by the headspace conditions under which the dough was mixed. For the dough mixed at ambient pressure, the velocity increased by about 60% of its original value when the pH was lowered to 4.3. Conversely, for the dough mixed under vacuum, the velocity decreased by 25% when the pH was lowered by the same amount.

Qualitatively, these two 'opposite' effects may be explained in terms of charge repulsion as follows. For the dough mixed at reduced pressure, there are few gas nuclei present in the dough. At the high pH value, there are many chain links (albeit weak) in the dough (Belton, 1999) and hence the rigidity of the dough is correspondingly large, so that a rather high velocity is measured (greater than water). At low pH values, where the side-chains of the ionizable amino acids are protonated, the proteins repulse each other, the links get broken and the matrix is softened. As a result, the sound velocity is smaller. A small reduction in dough density should also accompany the drop in pH value (Tanaka *et al.*, 1967), but the change in rigidity is by far the larger effect in determining the change in velocity for the vacuum mixed dough. On the other hand, for the dough mixed at atmospheric pressure, seven times as many air nuclei are present in the dough. These air nuclei have the dominant influence on sound propagation properties. As a result, the velocity is lower compared to the dough mixed under vacuum. At low pH, the same effect of side-chain protonation and repulsion of proteins will occur. However, because of the presence of a greater number of air nuclei, the repulsion of the proteins will compress the air nuclei thus causing them to shrink. Thus, dough acidification has two competing effects: weakening of the matrix and shrinkage of the air nuclei. Since for dough mixed at ambient pressure, the velocity is largely determined by the size and the number of the air nuclei at this low frequency (Leighton, 1997), shrinkage of the nuclei will dominate, resulting in an increase in velocity as the pH is lowered.

Even though these qualitative arguments provide an explanation of the behaviour of the velocity as a function of fermentation time, further research is needed to study the changes in the internal structure of the matrix resulting from the many chemical interactions that occur during fermentation. Evidently, the emphasis should be on the first 20 min of fermentation where the rate of production of CO_2 by the yeast has such a dramatic effect on ultrasonic velocity.

Table 1. Effect of pH on ultrasonic velocity (mean values ± standard deviation) in doughs mixed at atmospheric pressure and under vacuum.

Mixer headspace pressure (atm)	Dough pH	Velocity ($m\,s^{-1}$)
1.0	5.2	100 ± 5
1.0	4.3	164 ± 15
0.13	5.2	1955 ± 40
0.13	4.3	1480 ± 90

Ultrasonic Attenuation as a Function of Fermentation Time

The standard procedure for measuring the attenuation coefficient α is to first measure the ultrasonic signal amplitude as a function of sample thickness, and then to fit the data to an exponential decay. For dough without yeast, this method has been shown to give reliable results (Elmehdi, 2001). However, this procedure could not be used for the yeasted dough because fermentation evolved too rapidly for ultrasonic measurements to be made on different thicknesses of the same sample. An alternative approach is to determine the relative attenuation rather than the absolute one, i.e. measure the change in the attenuation coefficient, $\Delta\alpha$, relative to the attenuation coefficient at the onset of fermentation. The relative attenuation, $\Delta\alpha$, still provides valuable information for understanding changes in the structure of the dough resulting from fermentation. The expression for $\Delta\alpha$ can be derived from the amplitude decay equation, (1), to give:

$$\Delta\alpha(t - t_1) = \frac{2}{L}\ln\left[\frac{A(t_1)}{A(t)}\right] \tag{3}$$

where $A(t_1)$ and $A(t)$ are the signal amplitudes at fermentation times t_1 and t measured after the end of mixing, with t_1 being the time of the first reading (approximately $t = 4$ min)*. The relative attenuation coefficient as a function of fermentation time for the two mixer headspace pressures

*Note that in equation (3), no account has been taken of the possible decrease in signal amplitude that can occur at $x = 0$ from a change in the acoustic impedance of the sample. For fermenting dough, where the velocity and density both decrease with time, equation (3) will overestimate $\Delta\alpha$. Nonetheless, equation (3) still suffices to establish qualitative trends in the change of attenuation of fermenting dough.

is shown in Figure 6. The attenuation coefficient is seen to increase significantly during the first 30 min of fermentation for both doughs. After that, $\Delta\alpha$ becomes roughly constant, although after about 40 min the signal amplitude becomes small and difficult to measure (open symbols). Thus it may be surmised that the attenuation in both doughs is similar near the end of fermentation. The total change in $\Delta\alpha$ during fermentation is seen to be greater for the dough mixed under vacuum, reflecting the fact that the initial amplitude was larger.

To interpret these results, the change in attenuation should be expressed in terms of the change in the void fraction $\Delta\phi$ as fermentation proceeds. This enables the behaviour of $\Delta\alpha$ to be related to the degree of inflation of the gas bubbles in the dough. To do this, a fifth order polynomial was fitted to the density data in Figure 3, thus enabling the density to be determined for any value of the fermentation time. The change in void fraction was then calculated using the equation, $\Delta\phi = 1 - (\rho/\rho_{t_0})$, where the subscript in t_0 refers to 'zero fermentation time' (the end of mixing), and the values for ρ_{t_0} are 1240 and 1420 kg m^{-3} for the dough mixed at ambient pressure and under vacuum, respectively. The ρ_{t_0} values were determined by extrapolating the density data back to zero fermentation time using the polynomial fit.

The change in attenuation was then plotted as a function of change in void fraction (Figure 7). The change in attenuation coefficient increases with void fraction for doughs mixed at both pressures. However, for the dough mixed under vacuum, a sharp increase in $\Delta\alpha$ occurs initially even though the void fraction has not changed by much. This suggests that the diffusion of CO_2 affects the matrix in a way that increases the absorption of the ultrasonic signal in much the same way as it affects the velocity. This change in $\Delta\alpha$, which does not appear to be due to the increase in void fraction, is represented by the open symbols in Figure 7. In the region, $0.05 \leq \Delta\phi \leq 0.4$, the void fraction is changing rapidly because CO_2 has reached the air nuclei, and $\Delta\alpha$ increases approximately linearly with void fraction for both mixing pressures. Thus the change in attenuation is directly proportional to the void fraction in this range of $\Delta\phi$. Furthermore, the rate of change of the attenuation with change in void fraction is the same, within experimental error, for the doughs mixed at both pressures. This is shown by the solid lines in Figure 7, which both have slopes $\Delta\alpha/\Delta\phi = 6.2$. Although this slope is somewhat less than found previously for unyeasted dough (Elmehdi, 2001), where the attenuation was found to depend on void fraction as $\alpha = 0.245 + 9.8\phi$, in both cases the attenuation increases linearly with void fraction over most of the range of ϕ which are technologically important in dough processing operations.

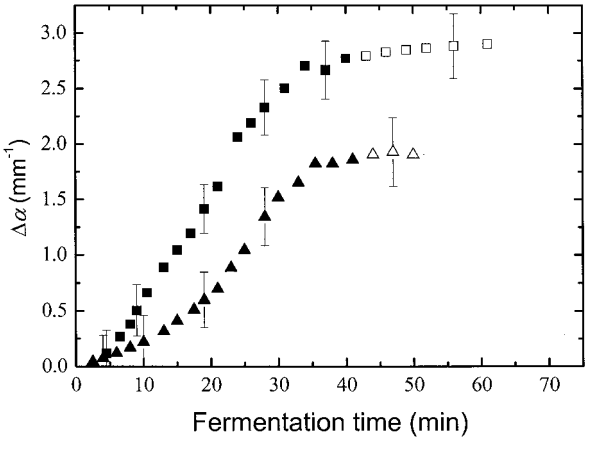

Figure 6. The change in the attenuation coefficient for fermenting dough mixed under vacuum (■) and at atmospheric pressure (▲). Open symbols represent data where the signal becomes very small and comparable to the noise level.

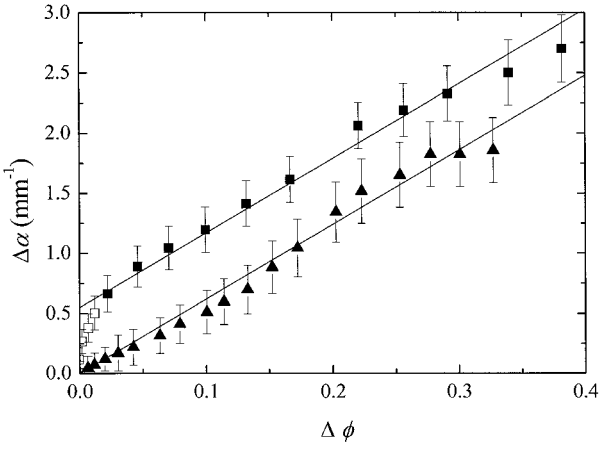

Figure 7. The change in the attenuation coefficient as a function of the change in void fraction for the dough mixed under vacuum (■) and at atmospheric pressure (▲). The solid lines show the approximately linear behaviour of $\Delta\alpha$, with slope $\Delta\alpha/\Delta\phi = 6.2$. The open symbols show the early fermentation time behaviour of $\Delta\alpha$ for dough mixed under vacuum, where the attenuation is strongly influenced by changes in the dough matrix.

CONCLUSIONS

The experimental results show that both the ultrasonic velocity and attenuation are sensitive to the presence as well as to the expansion of gas cells in the dough. The ultrasonic velocity decreased as the bubbles expanded, with a very much larger decrease in the case where the dough had been mixed under vacuum. By relating the change in the velocity to the corresponding change in the density during fermentation, it was found that there are two distinct mechanisms that contribute to the large changes in the velocity. The first is the dependence of velocity on the size and number density of the bubbles. The second mechanism, which is especially important at early fermentation times, arises from changes in the mechanical properties of the dough matrix. These changes occur as the dissolved CO_2 molecules diffuse through the matrix, causing a reduction in the pH and a weakening of the intermolecular interactions, likely due to charge repulsion effects on the proteins. Except at the early and later stages of fermentation, the change in ultrasonic attenuation was approximately linearly related to the degree of expansion of the gas cells, suggesting a new means of measuring these important breadmaking changes quantitatively. The results presented in this paper demonstrate the potential for using ultrasonic techniques to non-destructively characterize dough systems during processing operations, such as fermentation.

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