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ORIGINAL ARTICLE

Probing thermal transitions and structural properties of gluten proteins using ultrasound

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Abstract
Purpose To probe the thermal and structural properties of gluten proteins using ultrasound.
Methods A new ultrasonic approach for characterizing the quality of wheat gluten proteins is described. Low frequency (50 kHz) longitudinal ultrasonic velocity, v_L, measurements were performed on gluten samples extracted from three wheat flours differing in protein content and in wheat endosperm hardness.
Results At room temperature, v_L for gluten extracted from soft flowers (Fielder) was found to be (870 ± 92) m/s, while for gluten extracted from extra strong flours (Glenlea) it was found to be (1,940 ± 90) m/s. In the second set of experiments, which aimed at probing thermal properties of gluten proteins, the variation in the numerical value of v_L propagating in the wet gluten was found to be substantial (about 1,000 m/s) when the temperature of the gluten was raised from 20 to 90 °C, and also when gluten from different flour types was investigated. A continuous structural phase transition was observed, which was different for glutens extracted from different flours. Upon cooling, the velocity also varied depending on wheat type.
Conclusions These experiments demonstrate that ultrasonic velocity measurements can be used as a selection tool and study changes in properties of wheat proteins, particularly the thermal transitions that are critical to the quality of end products such as noodles, pasta, and bread. It was also shown that v_L is sensitive to gluten class (strength or protein content), showing the potential of such measurements as an early-generation selection tool in wheat breeding programs.

Keywords Ultrasound · Gluten proteins · Thermal transitions · Quality · Structure

Riassunto
Obiettivo Sondare le proprietà termiche e strutturali di proteine di glutine che usano l'ultrasuono.
Materiali e Metodi Un nuovo approccio ultrasonico per caratterizzare la qualità di proteine di glutine di frumento è descritta. La frequenza bassa (50 kHz) la velocità longitudinale ultrasonica, il v_L, le misure sono state eseguite sui campioni di glutine estratti da tre farine di frumento che differisce nel contenuto di proteina e nella durezza di endosperm di frumento.
Risultati Alla temperatura ambiente, il v_L per il glutine estratto dai fiori morbidi (l'Esterno) è stato trovato per essere (870 ± 92) il m/s, mentre per il glutine estratto dalle farine extra forti (Glenlea) è stato trovato per essere (1940 ± 90) il m/s. Nella seconda serie di esperimenti, che ha mirato a sondare le proprietà termiche di proteine di glutine, la variazione nel valore numerico di v_L che propaga nel glutine bagnato è stata trovata per essere sostanzioso (circa 1000 m/s) quando la temperatura del glutine è stata alzata da 20 a 90 °C ed anche quando il

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glutine dai tipi di farina diversi sono stati investigati. Una transizione di fase continua strutturale è stata osservata, che era diverso per i glutini estratti dalle farine diverse. Sul raffreddamento, la velocità il tipo di frumento di dipendere da anche vario.

Conclusioni Questi esperimenti dimostrano che le misure di velocità ultrasoniche possono essere usate come uni cambiamenti di attrezzo di selezione e studio nelle proprietà di proteine di frumento, particolarmente le transizioni termiche che sono critico alla qualità di prodotti finiti come i sempliciotti, la pasta ed il pane. È stato anche mostrato che il v_L è sensibile alla classe di glutine (il contenuto di forza o proteina), mostrando il potenziale di tali misure come un attrezzo di selezione di prima-generazione nei programmi di allevare di frumento.

Introduction

Gluten proteins, representing the major protein fraction of the starchy endosperm, are predominantly responsible for the unique position of wheat amongst cereals. Specifically, they are characterized by their ability to form a cohesive viscoelastic material called gluten when flour is mixed with water. Due to these physical attributes the resulting dough is able to retain gas bubbles during fermentation and baking, leading to baked products with high loaf volume and an appealing crumb structure.

The breadmaking potential, as well as the potential for producing high-quality noodle and pasta products, varies within a broad range for different wheat flours, and depends on both the genotype (variety) and the growing conditions (soil, climate, location, fertilization, etc.). Differences in wheat flour properties are mainly due to variations in the structure, amount and proportion of the different gluten proteins [1]. Gluten proteins may be classified, according to solubility behavior, as gliadins and glutenins. Gliadins consist of three different protein types, the ω-, α- and γ-gliadins [2, 3], and are responsible for the extensible characteristics of gluten. Glutenins exist as massive polymers linked together by intermolecular disulfide bonds and non-covalent forces and they determine gluten strength and elasticity. The glutenins can be subdivided into the very high molecular weight glutenins (HMW-GS) and the lower molecular weight glutenins (LMW-GS). Although both types of proteins are important, the HMW-GS have been directly related to the quality of leavened bread, pasta, noodle, and related products [4, 5].

Defining protein/gluten quality has proven to be a difficult task as several technologies have historically been utilized and recognized by various groups within the research and development community as well as in industry. Use of instruments such as the alveograph, extensigraph, farinograph, mixograph, texture analyzer [6–10], etc. to evaluate protein or gluten quality has had proponents and detractors.

One "new" technique that has been developed and has shown great potential for evaluating the structural properties of viscoelastic materials is low-intensity ultrasound [11–13]. By subjecting the sample to low-intensity longitudinal ultrasonic waves, information about the mechanical and structural properties of the sample may be readily extracted. Adapting the ultrasonic technique as a non-invasive and non-destructive system to evaluate the properties of gluten proteins extracted from different flour types (even from within the same class) may result in technical and economical advantages over existing wheat quality evaluation methods. This ultrasonic method has the potential to be more reliable, to supply fundamental rheological information to guide processors in bread dough as well as noodle and pasta manufacturing, and, because of its ability to utilize small samples, to be suitable for implementation in wheat breeding programs by providing quality information at an early stage. We therefore hypothesize that ultrasound can be used to discriminate glutens prepared from wheat of different classes and study the thermal profiles for gluten extracted from different wheat classes. In this paper, we will examine this hypothesis by presenting the results of extensive ultrasonic experiments performed on several proteins extracted from different wheat classes. Our results will be correlated with measurements made using conventional methods.

Materials and methods

Gluten samples

Flour samples were milled from wheats covering a range of dough strengths and protein content. A list of these samples along with their properties is presented in Table 1. The wheats were field-grown in different locations across the provinces of Manitoba and Saskatchewan, Canada. The samples were tempered between 15 and 16.5 % moisture content (depending on grain hardness) and milled using a Buhler laboratory mill at the Canadian International Grains Institute (CIGI) pilot mill (Winnipeg, Manitoba, Canada). Table 1 also summarizes the gluten varieties investigated in this paper, classified in terms of the wheat flour types from which the gluten samples were extracted. The first category is hard red spring wheat, which usually has the highest wheat protein content, and is used for bread, hard baked goods, all-purpose flour, and flour blends [14–16]. The sub-categorization of extra strong and strong is determined from the protein quality, but there are difficulties in adequately mixing the extra strong type doughs.

Table 1 Wheat types and their flour protein contents

Variety	Wheat type	Classification	Flour protein content %	Insoluble gluten content %	Origin
Glenlea	Hard red spring	Extra strong	12.5†	33†	Canada
Neepawa	Hard red spring	Strong	13.4†	23.5†	Canada
Fielder	Soft white spring	Soft	10.2†	16†	Canada
AC Corrine	Hard red spring	Extra strong	12.7††	–	Canada
AC Reed	Soft white spring	Soft	10.9††	–	Canada
Falcon	Hard red winter	Strong	12.7††	–	Canada

† Kovacs et al. (2004)

†† Unpublished Communication (CRC, Agriculture and Agri-Food Canada, Winnipeg)

Hard winter wheat flour is milled from high protein wheats, and is used mostly for breads and all-purpose flour, and as an adjunct in other flours to increase protein content, but is not as good for breadmaking as hard red spring wheats. The soft white spring wheat flour is from low-protein wheats, but offers high yields to growers. It provides a white product for high-quality cakes, crackers, cookies, pastries, and some Asian-style noodles, and Middle Eastern flatbreads [14, 16]. It is not regarded as suitable for breadmaking. The measured protein content in each of these samples is presented in Table 1.

The gluten samples used in these experiments were extracted from the milled flours in three steps. First, a cohesive flour–water dough was formed by mixing 15-g samples with either distilled water only (0 % saline) or 2 % saline solution. Second, the starch was first washed out by hand for one minute under distilled water or 2 % saline solution depending on the treatment. After that, the gluten sample was transferred to a Glutomatic instrument (2100, Perten Instruments AB, Huddinge, Sweden) for a final wash of 5 min. The extracted gluten was then immediately transferred into the ultrasonic equipment to measure the transit time. For gluten samples that were subjected to a cooking treatment, the gluten was heated for 90 s at the boiling temperature of water, and then allowed to cool down to room temperature. After that, the gluten samples were taken for ultrasonic measurements.

For all three treatments, the extracted gluten samples were sandwiched between the ultrasonic transducers (generator and receiver) forming a circular disc of specific thickness. As we will discuss in the experimental section, the thickness of the samples was accurately controlled using three micrometer heads (made by ULTRA PRÄZISION MESSZEUGE, Germany), which were fixed to the sample holder at 60^{o} angles.

Ultrasonic experiments

The transmission of ultrasound through a multi-phase material such as gluten is influenced not only by the properties of the various phases in isolation, but also by the heterogeneous physical structure into which the proteins are assembled. These structural features include the concentration, size, and distribution of phases or particles, and ultrasound sensitivity to these features depends on the mismatch in the acoustic properties of the constituents. The full picture is very complex but the corollary is that the ultrasonic response is sensitive to many of the key structural and mechanical properties. Therefore, there is considerable interest in using ultrasound in the food industry to monitor food properties, provided that the samples are otherwise well characterized [11–13, 17–19].

A longitudinal ultrasonic pressure wave, propagating through a fluid medium in the x-direction with a pressure field, ψ, is described by [20, 21],

$$\psi = \psi_0 \exp(-\alpha x/2)\exp(i[kx - \omega t]) \quad (1)$$

Here ψ_o is the pressure field at $x = 0$, α is the attenuation coefficient in m^{-1}, $k = 2\pi/\lambda$ is the wave number, λ is the wavelength, and ω is the angular frequency (=2 π f). It should be noted that the amplitude attenuation coefficient is defined as the intensity attenuation coefficient divided by 2, hence the factor 1/2 in Eq. (1). For more details on the difference between amplitude attenuation coefficient and intensity attenuation coefficient, the reader may consult Ensminger [20] and Liley [21]. The phase velocity at which the sound travels is$v = \omega/k$. In materials where attenuation is not too large, the phase velocity of longitudinally polarized waves is related to the longitudinal modulus, β, of the material and its density, ρ, by

$$v_l = [\beta/\rho]^{1/2} \quad (2)$$

where in a solid material, or a viscoelastic material such as gluten

$$\beta = B + \frac{4}{3}\mu. \quad (3)$$

Here B is the bulk modulus (equal to the inverse of the compressibility), μ is the rigidity modulus (shear modulus), and the subscript l in Eq. 2 denotes longitudinal.

The features of ultrasonic wave propagation of which most use has been made experimentally are the velocity and attenuation of the wave. For most purposes, it is only necessary to appreciate that a measurement of the ultrasonic velocity provides information about the ratio of an elastic modulus to the density of the material through which it propagates. Thus, independent measurements of density and velocity enable a value of the combined elastic modulus to be determined. Whereas knowledge of the wave speed provides information about the modulus of the material, the attenuation coefficient depends on other material properties; even in pure homogeneous materials, there are many possible causes of attenuation [11].

Experimental set-up and velocity measurements

A block diagram of the apparatus used for evaluation of the properties of wet gluten is shown in Fig. 1. A Portable Ultrasonic Non-destructive Digital Indicating Tester PUNDIT 6, made by CNS Farnell, was used to generate a short (+ve) voltage electromagnetic (EM) pulse (or spike). The EM pulse generator was operated at an EHT voltage of either 1.2 or 500 mV, as selected by a switch at the back of the unit, and it has the ability to output pulses at a repetition rate of either 10 pulses per second (pps) or 100 pps. A 3.5 V positive pulse with a rise time of 2 μs, synchronized with the main output signal, was used to trigger the oscilloscope. The generated ultrasonic signal traveled through the sample and was detected at the other face of the sample with a similar transducer, which converted the transmitted ultrasonic signal into an EM signal. This EM signal was then amplified at the receiver amplifier (PUNDIT 6) and displayed on a digital oscilloscope (Tektronix TDS 420 A).

The data were acquired using a computer-controlled digitizing oscilloscope (Tektronic TDS 420 A), which was set in averaging mode. The signal averaging, which consisted typically of 1,000 sweeps, greatly improved the signal-to-noise ratio. The triggering of the sweeps was performed by the time base (TB) synchronization output on the pulse generator, so as to synchronize the data acquisition with each repetition of the pulse from the signal generator. The acquired waveforms were transmitted directly to the hard disk of the computer for subsequent analysis.

The velocity and the amplitude were calculated from the waveforms that were stored in the computer, using computer software called Microcal Origin (Microcal Software Inc.). The amplitude of each waveform was directly measured from the height of the second oscillation in volts. The reason for using the second oscillation rather than the peak of the waveform was to avoid interference effects that arise from the ringing of the transducer and possible scattering effects. The velocity on the other hand was measured by calculating the time taken for the signal to travel from one side of the sample to the other. This was done in two steps. In the first step a reference waveform was acquired. This waveform was taken with the two transducers separated by a material of well-known acoustic properties. After the transmitted signal was acquired, the two waveforms (reference and transmitted) were downloaded to the computer. The transit time was then calculated by measuring the time difference between the first two oscillations of the reference and the sample waveforms as follows. This was accomplished by aligning the two waveforms using the pulse shape as a guide. The delay time introduced by the reference signal (through the acrylic plates) was then subtracted from the measured time, δt, to give the time taken for the signal to travel through the sample, t.

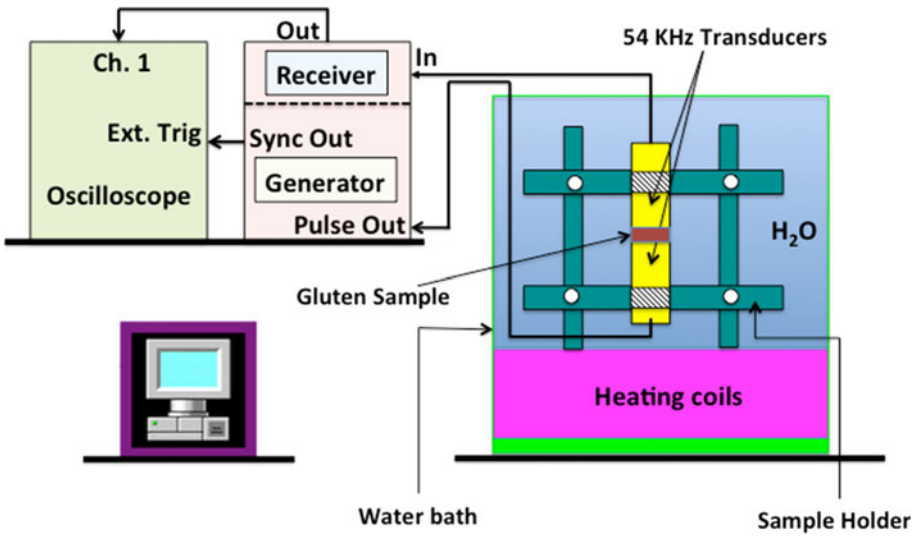

Fig. 1 Experimental set-up for measuring ultrasonic velocity in samples of wet gluten

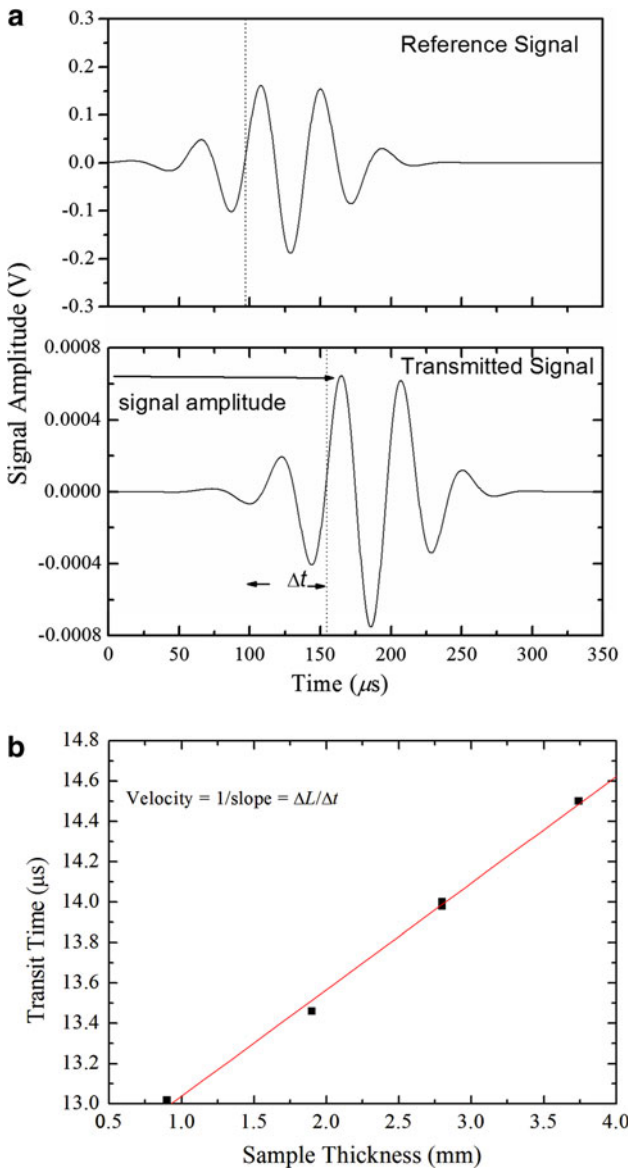

Fig. 2 **a** A typical example of the reference and transmitted signals used to measure the transit time. **b** The transit time through the sample as a function of sample thickness for sub-samples taken from the same gluten sample

Figure 2a shows an example of the reference, the transmitted waveforms and δt. The above procedure was repeated for several sample thicknesses ranging from 1 to 5 mm, with all gluten samples being washed from the same flour. After that, the transit time t was plotted *versus* the sample thickness generating a linear behavior. The velocity is then simply the inverse of the slope of the straight-line fit, i.e.,

$$v = \frac{1}{\text{slope}} = \frac{\Delta d}{\Delta t} \tag{4}$$

where d is the sample thickness. A typical example of the velocity calculations from the slope is given in Fig. 2b.

It should be noted that since the structure of the gluten protein samples is sensitive to temperature fluctuations, the gluten sample (along with the sample holder) was immersed in a water bath, allowing the temperature to be maintained within 0.1 of a degree, see Fig. 1. This should not affect the measurements since the water intake of gluten is negligible at the low temperatures used in these experiments [23]; this will be shown in a subsequent section where the density of the gluten as a function of temperature is discussed.

Results and discussion

Experiment one: using ultrasonic velocity as a wheat breeding tool

Traditionally, the quality of wheat samples in a breeding program is determined using a battery of tests. The purpose of all of these tests is to predict how the wheat sample will mill, make leavened bread, flat breads or noodles. The number and kinds of tests employed are based on the generation of the genetic material being tested, with more tests being done on the more advanced lines within the breeding program. Initially, samples are merely screened for protein content, kernel hardness, and gluten strength. In later generations, tests using mixographs, farinographs and a bake test will be used to determine the functional quality of a wheat sample. Many of these predictive tests for quality are time consuming, expensive, and require a large amount of grain. A fast, economical screening test that would permit many samples to be efficiently evaluated is therefore highly desirable. The use of ultrasound as a quality screening technique may satisfy these requirements.

In this section, ultrasonic velocity is used as a screening test for samples subjected to three different preparation treatments. In the first treatment, the gluten samples were prepared using distilled water after which they were immediately transferred to the testing apparatus. In the second treatment, the samples were washed using a 2 % NaCl solution and, similar to the first treatment, were immediately tested. In the third treatment, the samples were washed under a 2 % saline solution and then cooked for 90 s. The purpose of the three treatments was to investigate the effect on the ultrasonic parameters of the gluten rearrangement brought about by the addition of NaCl and elimination of some of the associated gluten bonding effects due to charge screening of the gluten polyelectrolyte by the counter-ions.

The effects of the three gluten preparation treatments on ultrasonic velocity are shown in Fig. 3 and are summarized in Table 2. The reported velocity values reported in Table 2 represent the computed mean of five replicates,

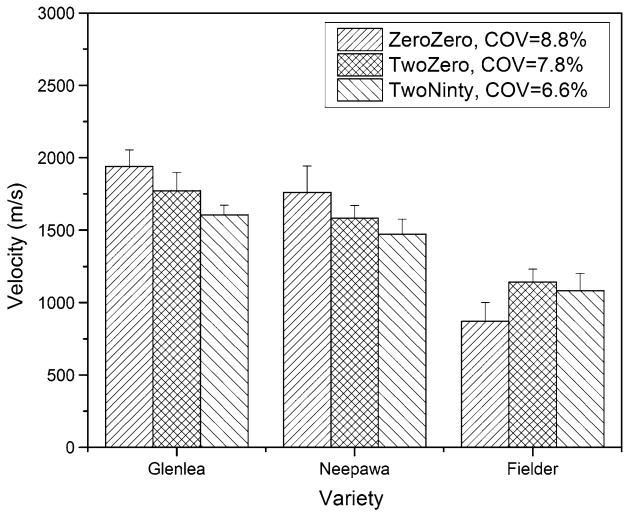

Fig. 3 The ultrasonic velocity in gluten made from three wheat varieties according to three treatments. The coefficient of variation (c.o.v.) is shown for each treatment

and the associated uncertainties were calculated from the standard deviation. It should be noted that the reported values of the velocities were performed at room temperature. For statistical purposes, each measurement has been repeated at least five times from different sub-samples of the same gluten extract from the same flour, so that the mean value along with the standard deviation as well as the p value could be determined.

The variation in the ultrasonic velocity with flour type is striking. It is clear from these results that the magnitude of the ultrasonic velocity for the stronger variety (Glenlea) is higher than the weaker one (Fielder) by about 1,000 m/s. Differences in ultrasonic velocity at this frequency for dough samples (rather than wet gluten) have previously been reported [22], with velocities in doughs prepared from harder wheats being greater than those from softer wheats (although differences in velocity were not as pronounced as those reported for gluten samples here). It should also be noted that as the treatment of the gluten was changed so did the magnitude of the ultrasonic velocity, indicating that both NaCl concentration and the cooking process influence the gluten structure that is monitored by the ultrasonic signal propagation. In fact, the coefficient of variation was found to be the smallest (6.6) for the third treatment, i.e., washing with 2 % NaCl solution and 90 s cooking time. This result is not unexpected since the effect of the addition of NaCl is to "order" the structure of the gluten. These results indicate that ultrasound velocity in gluten prepared under all three conditions may indeed be used to differentiate between gluten extracted from different flour varieties. The p values for each set of the reported velocities for each treatment condition were computed using a two-tale test with 95 % confidence interval level. For all three cases the p value was <0.05 %.

Linear correlations between ultrasonic velocity and other screening tests, such as measurement of viscoelasticity of heated gluten (visco), sedimentation of proteins in the surfactant SDS (SED), which is an indicator of end use quality [24], mixograph development time (MDT) and total energy (TEG), and farinograph dough development time (DDT) were performed to compare the results of the ultrasonic experiments and the listed tests, which are routinely used to study gluten proteins. Table 3 summarizes the linear correlation coefficient between our ultrasonic velocity measurements and each of the other tests listed above. It is evident from Table 3 that the linear correlation coefficients were low for treatment one (0 % salt and zero cooking time), varying from 0.45 to 0.65. The linear correlation coefficient is higher for treatments two and three, reaching as high as 0.99 for treatment three. This is an indication that the presence of salt modifies gluten properties in such a way that ultrasonic velocity correlates better with parameters derived from traditional flour quality tests. For the other two treatments, where 2 % NaCl was added, correlations with standard wheat quality screening tests were very good, with an R value for some tests as high as 0.99 (SED). Because of the dramatically improved relationships between the denatured gluten samples and the conventional quality indices, we investigated whether there were changes in the ultrasonic properties of gluten samples during thermal denaturation.

Table 2 Summary of ultrasonic velocity measurements for gluten extracted from different wheat varieties using three treatments

Variety	Velocity (m/s)*,† treatment 1 (zero NaCl and zero cooking time)	Velocity (m/s)*,† Treatment 2 (2 % NaCl and zero cooking time)	Velocity (m/s)*,†† Treatment 3 (2 % NaCl and 90 s cooking time)
Glenlea	1,940 ± 90	1,770 ± 97	1,600 ± 95
Neepawa	1,760 ± 93	1,580 ± 90	1,470 ± 95
Fielder	870 ± 92	1,140 ± 100	1,080 ± 98

* The reported velocity values represent the computed mean of 5 replicates and the associated errors were calculated from standard deviation

† Treatment 1 and 2 were performed at room temperature

†† Treatment 3 was performed at 90 °C

Table 3 Linear correlations of ultrasonic velocity with conventional empirical wheat quality screening tests

Screening test	Linear correlation coefficient of our ultrasonic measurements. Treatment 1: 0 % NaCl and zero cooking time	Linear correlation coefficient of our ultrasonic measurements. Treatment 2: 2 % NaCl and zero cooking time	Linear correlation coefficient of our ultrasonic measurements. Treatment 3: 2 % NaCl and 90 s cooking time
Visco	0.50	0.72	0.95
SED	0.51	0.68	0.99
MDT	0.49	0.64	0.83
TEG	0.45	0.71	0.86
DDT	0.56	0.60	0.87
Stability	0.62	0.64	0.9
MTI	0.65	0.92	0.98

Experiment two: denaturation and thermal phase transitions in gluten proteins

The effects of protein denaturation can be observed in several ways, for example, as a change in solubility [25] or as simultaneous changes in chemical, physical, and biological properties [26, 27]. These changes in physical, and to a lesser extent chemical, properties are manifestations of configurational changes taking place in the polypeptide chains. Most denaturation changes consist of changes in secondary bonds: ion–dipole, hydrogen and Van der Waals, and in the rotational positions about single bonds which are controlled by the secondary bond structure [28].

Although denaturation can be achieved by a number of methods such as raising the temperature [29, 30], changing pH [31], using various denaturant chemicals [31, 32], and high pressure homogenization [33], since the results reported in this paper deal with denaturation achieved solely with increasing temperature, the other methods will not be discussed further.

The temperatures at which various proteins unfold vary enormously [30]. Many proteins unfold at temperatures only a few degrees higher than those at which they function. Others, such as the gluten proteins, are stable to much higher temperatures. The driving force for denaturation is the increase in entropy that accompanies the transition of a single conformation into an ensemble of random ones [31]. The early unlocking of the tertiary structure deletes a large number of the bonds holding the structure together but increases the randomness only insignificantly. The later stages of denaturation lead to larger increases in entropy. Thus, the intermediate states are relatively unstable, and heat denaturation is often an all-or-none phenomenon. The unfolding of the protein exposes the buried non-polar amino acid residues, and their intermolecular clustering leads to aggregation of the denatured protein. Consequently, heat denaturation is essentially irreversible [30].

As noted above, the aim of this section of the paper is to show how ultrasonic techniques may be used to monitor the structural changes that accompany protein denaturation. To do that, the temperature of the water bath was increased gradually in increments of 1 °C. Before taking the reading, the gluten sample was allowed to reach equilibrium by sitting at the target temperature for a few minutes. This was confirmed in a separate experiment where the transit time was found to be constant after a few minutes. After the maximum temperature was reached (90 °C), the gluten sample was cooled down to room temperature. This maximum temperature was determined by the operational limitations of the transducers, which was set by the manufacturer. Three gluten samples were investigated, which were extracted from flour from three different Western Canadian wheat varieties: AC Corinne (extra strong), AC Reed (soft wheat), and Falcon (winter wheat).

The results of the ultrasonic velocity measurements for the three gluten types are shown as a function of temperature in Fig. 4. Similar overall behavior is observed for all three samples with the velocity increasing gradually as the gluten was heated. Structural phase transitions were observed, albeit with different ultrasonic signatures, for all three gluten samples. Changes in ultrasonic velocity in the pre-transition region (<30 °C) were slight for all gluten samples extracted from the different wheats. At the transition region (30–45 °C), the velocity was found to be independent of temperature. For the proteins extracted from the strong wheat flour, the velocity continued to increase in the post-transition region until the maximum temperature of 90 °C was reached. However, for the gluten extracted from the soft and winter wheat flours, the velocity reached a plateau that started at approximately 65 °C for Falcon and at 55 °C for Reed. The winter wheat gluten showed different behavior: the velocity has a minimum, characteristic of a continuous phase transition, at a lower temperature, 34 °C, after which the velocity increased to a maximum at 65 °C. Upon cooling, the velocity also varied depending on wheat type.

Changes in the mechanical properties of gluten induced by changes in protein structure due to a temperature rise have been studied by many researchers using different

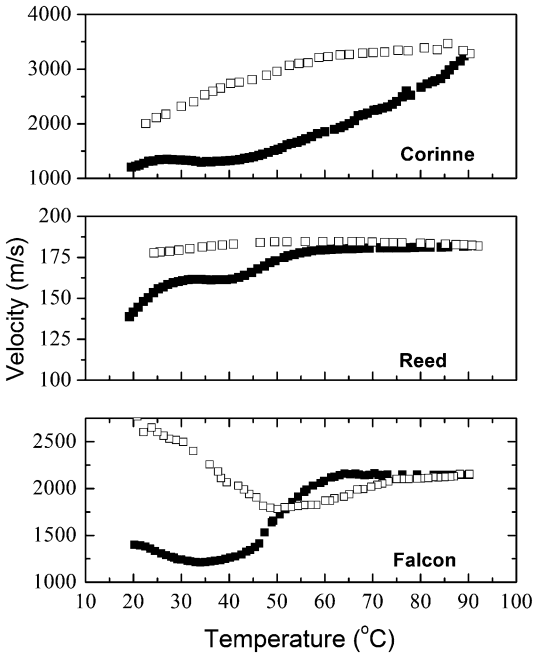

Fig. 4 The velocity of low frequency ultrasound in the three types of gluten proteins examined. Note the difference in the velocity scales. The *solid symbols* represent the velocity as the gluten was heated up, and the *open symbols* represent the velocity upon cooling down

techniques. For example, Hayat and Schofield [34] showed that hydrated wheat gluten behaved typically as a visco-elastic material in small strain dynamic shear tests with a storage modulus greater than the loss modulus. They also found a large change in the rheological behavior in the temperature range 30–90 °C, with evidence of greater network formation since the changes in the elastic modulus (G'$^{-}$) were considerably greater than the changes in the viscous modulus (G''). The source of this network formation was ascribed to di-sulfide cross-linking between gliadin proteins (Stathopoulos et al. 2006), since gluten proteins containing larger polypeptides (which are already cross-linked by di-sulfide bonds [5]) were comparatively less affected by the heat treatment. The effect of this thermally induced change on the mechanical properties of the intermediate size protein fractions heated up to 90 °C was a change in the dominant modulus from viscous to elastic. On the basis of changes in proteins induced by heating gluten dispersions at high temperature, it has been concluded that the gliadins are cross-linked into the glutenin structure [35, 36]. Georget and Belton [37] observed significant changes in the secondary structure of gluten proteins during heating and cooling cycles that would be expected to alter the bulk and/or shear modulus of the gluten, and thus cause the velocity of ultrasonic propagation to change as observed in these experiments.

At the end of the cooling stage, the magnitude of the velocity difference between the native state and the denatured state varied according to wheat sample. This difference may relate to technological quality factors, e.g., it may explain the ability of doughs to resist rupture during ovenspring, a result compatible with variability in shear moduli according to wheat variety following denaturation [38, 39]. Another observation from Fig. 4 is that the room temperature velocity for Reed, Corinne and Falcon was 260, 1,390, and 1,400 m/s, respectively. This result may be used by breeders to differentiate between flour varieties in accordance to their breadmaking strength [39].

It should be noted here that all gluten samples contain air bubbles, which are known to influence the propagation of the acoustic wave. These air bubbles were introduced to the gluten matrix early during the mixing process and later during the final stage of washing in the Glutomatic instrument [40]. The effect is more pronounced when the operating frequency is near the resonance frequency. For a multi-phase material such as gluten, resonance scattering may occur if the density of the dispersed phase (bubbles) is much less than the continuous phases (gluten) [40, 41]. Near the resonant frequency, the ultrasonic velocity peaks. Below the resonant frequency, the velocity is considerably less than its value in the surrounding matrix and can also be less than the value in the gas inside the bubbles [42]. At frequencies above the resonant frequency, the velocity is expected to be the same as that of the matrix. In previous work, the authors have shown in similar ultrasonic studies on flour–water bread dough, in which measurements were performed at similar frequencies, that the velocity is lower than the velocity of sound in air, corresponding to the situation well below the resonant frequency of the bubbles [17]. The reason for such a result is due to the enhanced compressibility arising from the presence of the bubbles [43]. Several researchers, including the authors, have examined the effect of bubble size and concentration on the propagation of acoustic waves in soft materials containing bubbles (see for example ref [11, 44]). These researchers have shown that ultrasonic velocity is sensitive to the presence of the bubbles at low frequencies, and to the property of the matrix at high frequencies. Thus, large differences in the velocity can be observed in experiments at 50 kHz depending on the bubble sizes, even when the concentration of bubbles is very low, consistent with the large differences seen in the experiments on gluten from different flour types.

The density of the gluten samples was measured to inspect its contribution to the observed transitions in the velocity as the temperature increased. To do this, samples were placed in a specific gravity bottle and the volume of the displaced liquid was measured. After correcting for the expansion of water, the density, ρ, was calculated using $\rho = m/V$, where m is the mass and V is the volume of the gluten sample. The density results are shown in Fig. 5. It is

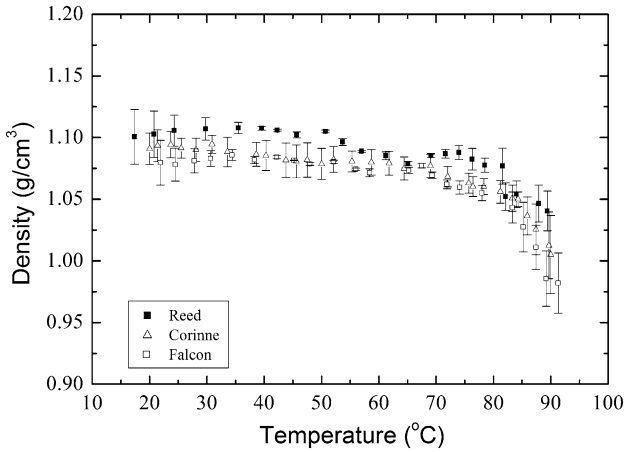

Fig. 5 The density of gluten as a function of temperature for the three samples examined in Fig. 4

clear that the density of the gluten samples stays roughly constant for temperatures up to 80 °C. After that, the density decreases, perhaps due to the expansion of the air bubbles within the gluten as the boiling point of water is approached. The temperature independence of the density is an important result. It shows that the observed changes in ultrasonic velocity involve the realignment of the polymers without any net thermal expansion. Thus, the rearrangements of the molecules act more like a shear alignment than a compressive or extensional rearrangement. It should also be noted that the geometry of the gluten samples used in the ultrasonic velocity measurements and the density measurements is different, and the sandwiching between the transducers limits the amount of gluten surface exposed to water in the ultrasonic experiments. In the ultrasonic measurements, the samples were disc-shaped of radius which is same as the transducer radius (1 in.), and width determined by sample thickness. Gluten exposure to the water would be limited to the perimeter of the disc. For the density measurements, the samples were spherically shaped. Therefore, the water intake is expected to be higher for the samples used in the density measurements, while for the samples used in the ultrasonic experiments, where the diffusion path is longer, the water intake is expected to be negligible as suggested above.

Conclusions

The work presented in this paper may be divided into two areas: investigating the mechanical properties of gluten proteins extracted from wheats of different strengths (1) at a single temperature (room temperature) and (2) as a function of temperature (as the protein denatures). In both experiments, low frequency (50 kHz) ultrasonic velocity was employed. The motivation for this work is the need to better understand the key role played by structural transitions and mechanical properties of gluten proteins so as to develop new ultrasonic techniques for characterizing the physical properties of these systems.

Our experimental results have shown that the ultrasonic velocity is sensitive to the structure and mechanical properties of gluten proteins. We exploited this phenomenon to investigate the effect of heating the gluten samples, so that the changes that accompany gluten protein denaturations could be studied. We found that the ultrasonic velocity is indeed sensitive to thermal transitions that occur when the gluten proteins are heated to temperatures above the denaturation temperature. These results demonstrate that ultrasonic techniques can be used to measure changes in physical, chemical, and biological properties of wheat proteins, which relate to noodle and pasta quality as well as baking quality. The sensitivity of ultrasound to measured differences between gluten from different cultivars shows its potential to be used as an early-generation selection tool for plant breeders.

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Conflict of interest Hussein Mohamed Elmehdi, Martin G. Scanlon, John H. Page, and Miklos I. P. Kovacs declare that they have no conflict of interest.

Informed consent No patient information was included in this study.

Human and animal studies The study described in this article did not include any procedures involving humans or animals.

References

1. Du Cros DL (1987) Glutenin proteins and gluten strength in durum wheat. J Cereal Sci 5:3–12
2. Shewry PR, Tatham AS, Forde J, Kreis M, Miflin BJ (1986) The classification and nomenclature of wheat gluten proteins: a reassessment. J Cereal Sci 4:97–106
3. Shewry PR, Halford NG, Belton PS, Tatham AS (2002) The structure and properties of gluten: an elastic protein from wheat grain. Philos Trans R Soc Lond B Biol Sci 357:133–142
4. Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987) The relationship between HMW glutenin subunit composition and the breadmaking quality of British-grown wheat varieties. J Sci Food Agriculture 40:51–65
5. Shewry PR, Halford NG, Tatham AS (1992) The high molecular weight subunits of wheat glutenin. J Cereal Sci 15:105–120
6. Kovacs MIP, Dahlke G, Noll JS (1994) Gluten viscoelasticity: its usefulness in the Canadian durum wheat breeding program. J Cereal Sci 19:251–257
7. Kovacs MIP, Poste LM, Butler G, Woods SM, Leisle D, Noll JS et al (1997) Durum wheat quality: comparison of chemical and rheological screening tests with sensory analysis. J Cereal Sci 25:65–75

8. Wang C, Kovacs MIP (2002) Swelling index of glutenin test. I. Method and comparison with sedimentation, gel-protein, and insoluble glutenin tests. Cereal Chem 79:183–189
9. Wang C, Kovacs MIP (2002) Swelling index of glutenin test. II. Application in prediction of dough properties and end-use quality. Cereal Chem 79:190–196
10. Uthayakumaran S, Newberry M, Phan-Tien N, Tanner R (2002) Small and large strain rheology of wheat gluten. Rheol Acta 41:162–172
11. Povey MJW (1997) Ultrasonic techniques for fluid characterization. Academic Press, San Diego
12. Létang C, Piau M, Verdier C, Lefebvre L (2001) Characterization of wheat-flour-water doughs: a new method using ultrasound. Ultrasonics 39:133–141
13. Elmehdi HM, Page JH, Scanlon MG (2004) Ultrasonic investigation of the effect of mixing under reduced pressure on the mechanical properties of bread dough. Cereal Chem 81:504–510
14. Beuerlein J (2001) Classes and Uses of Wheat Fact Sheet. http://ohioline.osu.edu/agf-fact/0146.html
15. WholeHealthMD. http://www.wholehealthmd.com/ME2/Default.asp
16. US Wheat Associates (2005) Wheat Classes. http://www.uswheat.org/uswPublic2009.nsf/index?OpenPage
17. Elmehdi HM (2001) An ultrasonic investigation of the effect of voids on the mechanical properties of bread dough and the role of gas cells in determining the cellular structure of freeze-dried breadcrumb, Ph.D. Thesis. University of Manitoba, Canada
18. Elmehdi HM, Page JH, Scanlon MG (2003) Monitoring dough fermentation using acoustic waves. Trans Inst Chem Eng Part C 81:217–223
19. Scanlon MG, Elmehdi HM, Page JH (2002) Probing gluten interactions with low-intensity ultrasound. In: Wheat Quality Elucidation, the Bushuk Legacy. AACC Press, St Paul, pp 170–182
20. Ensminger D (1988) Ultrasonics: Fundamentals, Technology and Applications.Taylor & Francis Group, CRC Press, FL
21. Liley D. The Physics of Ultrasound. http://opax.swin.edu.au/~dliley/lectures/het408/ultrasound.pdf
22. Kidmose U, Pedersen L, Nielsen M (2001) Ultrasonics in evaluating rheological properties of dough from different wheat varieties and during aging. J Texture Studies 32:321–334
23. Gontard N, Ring S (1996) Edible wheat gluten film: influence of water content on glass transition temperature. J Agric Food Chem 44:3474–3478
24. Kovacs MIP (1985) An improved sodium dodecyl sulfate-sedimentation test for early generation screening of durum wheat quality. Sciences des Aliments 5:123–131
25. Mirsky AE (1941) Sulfhydryl groups of egg albumin in different denaturing agents. J Gen Physiol 24:709–723
26. Langmuir I (1938) Protein denaturation. Cold Spring Harb Symp Quant Biol 6:159
27. Neurath H, Greenstein JP, Putnam FW, Erickson JO (1944) The chemistry of protein denaturation. Chem Rev 34:157–265
28. Lumry R, Eyring H (1954) Conformation changes of proteins. J Phys Chem 58:110–120
29. Bull BH, Breese K (1973) Thermal transitions in proteins. Arch Biochem Biophys 156:604–612
30. Stellwagen E, Wilgus H (1978) Relationship of protein thermostability to accessible surface area. Nature 275:342–343
31. Wu YV, Inglett GE (1974) Denaturation of plant proteins. Related to functionality and food applications: a review. J Food Sci 39:218–225
32. Wolf WJ, Briggs DR (1958) Studies on the cold-insoluble fraction of the water-extractable soybean proteins. II. Factors influencing conformation changes in the 11 S component. Arch Biochem Biophys 76:377–393
33. Hinrichs J, Rademacher B (2004) High-pressure thermal denaturation kinetics of whey proteins. J Dairy Res 71:480–488
34. Kovacs MIP, Fu BX, Woods SM, Khan K (2004) Thermal stability of thermal gluten proteins: its effect on dough properties and noodle texture. J Cereal Sci 39:9–19
35. Hayta M, Schofield J (2004) Heat and additive induced biochemical transitions in gluten from good and poor breadmaking quality wheats. J Cereal Sci 40:245–256
36. Naeem HA, MacRitchie F (2003) Heat stress effects on wheat proteins during grain development. In: Proceedings of the tenth international wheat genetics symposium, vol 1, pp 455–458
37. Singh H, MacRitchie F (2004) Changes in proteins induced by heating gluten dispersions at high temperature. J Cereal Sci 39:297–301
38. Georget DMR, Belton PS (2006) Effects of temperature and water content on the secondary structure of wheat gluten studied by FTIR spectroscopy. Biomacromolecules 7:469–475
39. Stathopoulos CE, Tsiami AA, Dobraszczyk BJ, Schofield JD (2006) Effect of heat on rheology of gluten fractions from flours with different bread-making quality. J Cereal Sci 43:322–330
40. Campbell GM, Rielly CD, Fryer PJ, Sadd PA (1998) Aeration of bread dough during mixing: effect of mixing dough at reduced pressure. Cereal Food World 43:163–167
41. Gaunaurd GC, Uberall H (1981) Resonance theory of bubbly liquids. J Acoust Soc Am 69:362–370
42. Fairley P, McClements DJ, Povey MJW (1991) Ultrasonic characterization of some aerated foodstuffs. Proc Inst Acoustics 13:63–70
43. Leighton TG (1994) The acoustical bubble. Academic Press, London
44. Scanlon MG, Elmehdi HM, Leroy V, Page JH (2008) Using ultrasound to probe nucleation and growth of bubbles in bread dough and to examine the resulting cellular structure of bread crumb. In: Campbell GM, Scanlon, MG, Pyle DL (eds). Bubbles in Foods 2: Novelty, Health and Luxury. AACC Press, St Paul, pp 217–230