Influence of internal interfacial area on nanosecond relaxation of wheat gluten proteins as probed by broadband ultrasonic spectroscopy

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A B S T R A C T

Understanding interactions between interfaces and biopolymers in complex industrially processed materials of plant origin will allow for their better utilization. Wheat flour doughs are one such material whose industrial use strongly depends on such interactions due to their effect on the mechanical properties of the dough. To date, mechanical characterizations of dough have been limited to a narrow range of frequencies. Here, ultrasonic spectroscopy measurements over a very broad frequency range are used to show that a fast volumetric relaxation occurs in dough; the nanosecond timescale of the relaxation is associated with ultrasonic stress-induced changes in the secondary structure of gluten proteins. Interestingly, there is a four-fold difference in the speed of this relaxation phenomenon in doughs mixed in air (where substantial internal interfacial area exists) compared to those mixed under vacuum (where bubbles are absent). Given the large internal interfacial area in dough, the amphiphilic proteins residing at gas bubble interfaces significantly alter the high-frequency mechanical response of this important material.

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1. Introduction

The complex mixture of structured biopolymers extracted from wheat endosperm is one of our planet's great renewable resources [1,2]. Understanding the structure, properties and dynamics of the hydrated version of this resource is essential for more efficient conventional use of the material in a variety of foods [2–4], but also for its better exploitation in the manufacture of industrial products [2,5–7].

During conventional processing, the dough that is formed from refined wheat endosperm particles occludes a significant volume fraction of air [8–11]; its value varies with the gas pressure exerted on the dough surface [9,12], the viscosity of the dough [13–15], and the type and duration of the process [10,11]. In emphasizing the importance to end-product characteristics of gas entrapment by the viscoelastic network, it has been stated that "gas production and retention are the keys to breadmaking" [16]. Further evidence of the significance of gas-holding to end-use properties is the utilization of cutting-edge techniques, such as the European Synchrotron Radiation Facility, to understand gas-holding functionality [17–19].

As well as influencing end-product properties, the gas content and how the gas is distributed within the dough affect its physical properties [20–22]. A typical median diameter of air bubbles that is nucleated in a breadmaking dough is $100\,\mu m$ [23–25], so that with dough's typical volume fraction of air of 0.1, dough has a substantial internal interfacial area. An interfacial area to volume ratio of $1400\,m^{-1}$ is a typical figure for dough [26], a value that is not dissimilar to classically porous materials such as bone [27,28] and some porous ceramics [29]. In a theoretical examination of the influence of internal interfacial area on the mechanical properties of dough, it was concluded that 80% of the strain energy in a dough deformed to 4% strain arose from changes in dough's interfacial free energy [26].

An additional consequence of a large internal interfacial area is that the interface is a preferential location for the many of the surface-active biopolymers that are present in dough [30–32]. Such molecules have been assigned significant roles in the end-use performance of products made from dough [31,33,34], but few studies have investigated the mechanical significance of these surface-active molecules. This contrasts with highly aerated food systems, where numerous studies examining the link between the mechanical properties (rheology) of foams and the type of surface-active biopolymer [35–37], its conformation at the interface [35,38,39], or its dynamics [36,40] have been conducted. In such investigations, spectroscopic techniques are intensively used tools;

pertinent examples are circular dichroism, Raman optical activity and polarization-modulation infrared reflection–absorption spectroscopy being used to probe the secondary structures of protein at an interface [35,38], the conformational stability of wheat proteins [41] and the synergy in the interfacial adsorption between surface-active proteins [42], respectively.

In addition to being effective at probing the effect of gas bubbles on the mechanical properties of biological materials [12,22], ultrasound has a long history as a spectroscopic technique for determining the mechanical response of polymer molecules at high frequencies [43]. By utilizing ultrasound over a wide range of frequencies, a variety of interesting phenomena that operate at different time scales can be interrogated. Given the broad distribution of relaxation times that is evident in dough [44–46], the insights from high frequency assessments of mechanical behavior allow a fuller characterization of dough's mechanical response and how this response is affected by the internal interfaces in the dough. However, to date there have been few high frequency investigations of dough properties, in large part because the highly absorptive nature of wheat flour dough makes accurate measurements of ultrasonic velocity and attenuation experimentally challenging [22,47]. In particular, determining the mechanical response of dough's hydrated viscoelastic network is hindered by strong ultrasonic absorption associated with the broad resonance peak arising from the polydisperse distribution of bubbles that is present in the dough [22,48].

The purpose of this paper is to report on ultrasonic spectroscopy measurements of wheat flour dough (made without yeast) using longitudinally polarized pulses over a wide frequency range (50 kHz to 30 MHz, by far the broadest frequency range yet reported). To ascertain how the large interfacial area associated with the gas bubbles in dough affects its mechanical response, dough was also mixed under vacuum to provide a reference material in which the internal interfacial area associated with these bubbles is minimized.

2. Experimental

2.1. Materials

Doughs were prepared from 100 g of a strong breadmaking flour (CWRS) that had been milled on the CIGI pilot mill (Winnipeg, MB, Canada). Reagent grade (Fisher Scientific, Nepean, ON, Canada) sodium chloride (2.4 g) was dissolved in 61 ml of distilled water that was then mixed with the flour. A high-speed pin-mixer, specifically a GRL-200 recording mixer [49], operating at 225 rpm, was used to mechanically develop the dough's viscoelastic network. Doughs were mixed for 5.5 min, the optimal mixing time as assessed from a torque vs mixing time curve when dough was prepared at atmospheric pressure.

To mix doughs under vacuum, the GRL-200 mixer was coupled to a vacuum pump via a digital pressure gauge and control valve to record and control headspace pressure during mixing. Vacuum grease was used to seal the mixing bowl. Ingredients were identical to those of the dough mixed at atmospheric pressure, but they were mixed for 1 min before evacuation to allow the flour to hydrate so that flour particles would not be drawn out. Vacuum mixing was therefore conducted for the last 4.5 min of mixing for a total time of 5.5 min.

After mixing, the dough piece was stored in a sealed container. Subsamples were cut from the dough piece with scissors; size was chosen according to the thickness to which the subsample was to be compressed. Subsample mass was determined from the difference of the mass of the container with the dough piece before and after excising the subsample.

Subsamples were also removed to determine dough density by weight in air and weight in water measurements [11,12].

2.2. Ultrasonic measurements

Two sets of apparatus were used for ultrasonic transmission measurements: one for low and very high frequency measurements (40 kHz, 20 MHz), and the other for high frequency measurements (500 kHz and greater). Block diagrams are shown in Fig. 1, with all connections made with 50 Ω BNC cables. Pairs of ultrasonic transducers were purchased from Panametrics (Olympus NDT, Waltham, MA).

In the low-frequency set-up, transducers were nominally 50 kHz, but their central frequency was ascertained as approximately 40 kHz. In the high frequency experiments, various transducer pairs of different central frequencies were employed (1.0, 2.25, 5.0 and 20 MHz). Two sets of transducer holders were used to contain the dough subsamples: direct contact and acrylic plate delay measurements. Direct contact between dough and transducers was used for the majority of measurements.

In the contact measurement holder, three micrometers, with a range of 28 mm, dictated the separation of the two steel plates (and thus the separation between the transducers). The dough subsample was compressed to the desired separation by manipulating the three micrometers. Securing screws were then set to maintain plate separation and hence sample thickness (Fig. 1a).

The acrylic plate delay measurement holder was used for the higher frequency measurements of doughs that had been mixed at atmospheric pressure (500 kHz to 5 MHz, but not the 20 MHz measurements). Copper plates (each 0.30 mm thick) were inserted between the acrylic plates to control dough subsample thickness. Two transducers, secured by clamps that allowed transducer alignment to be adjusted, were bonded via coupling gel to the acrylic plates (Fig. 1b).

In order to make the area covered by the compressed subsample essentially identical at different thicknesses, the mass of the subsample was selected for the low frequency measurements by:

$$m = \pi R^2 d\rho, \tag{1}$$

where R is the desired radius of coverage, d is the thickness of the subsample that was analyzed, and ρ is dough density. To ensure that the dough covered the whole surface of the transducer, a value of R (2.7 cm) was selected that was larger than transducer radius (2.2 cm). For the high frequency measurements, sample thickness was thin (<1 mm), and so subsample size was chosen by experience with the aim of attaining good transducer surface coverage. The circumference of both holders was sealed with tape to prevent moisture loss during ultrasonic measurements.

2.3. Analysis of ultrasonic data

2.3.1. Analysis in the time domain

The low (40 kHz) frequency transducers had a narrow bandwidth, and it was assumed that dispersion of ultrasound in the dough would not occur over this narrow frequency range. Plots of the transit time of the first oscillation minimum and the amplitude of the first oscillation maximum as a function of sample thickness permitted the effects of impedance mismatches to be eliminated so that ultrasonic velocity and attenuation could be accurately determined [12].

Where only one subsample thickness was used, or where time evolution observations of a single sample were desired, a reference signal was used in conjunction with sample measurements for ultrasonic velocity and attenuation determination. The reference signal was measured with the two transducers in direct contact (i.e., in the absence of the sample), or with a material