

# Use of Ultrasound to Investigate Glucose Oxidase and Storage Effects on the Rheological Properties of Cooked Asian Noodles

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## ABSTRACT

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This is the first use of a longitudinal ultrasonic technique to address the rheological properties of cooked noodles. Ultrasound (11 MHz) was utilized to investigate the influence of glucose oxidase (GOx) at the 1.5 U/g of flour level on the rheological properties of cooked alkaline noodles before and after 72 h of storage at 4°C. Cooked noodle dough samples were studied by simultaneously conducting stress relaxation and transmission ultrasonic measurements, yielding Peleg's  $K_1$  and  $K_2$  parameters (initial rate of relaxation and extent of relaxation, respectively) and ultrasonic information on noodle texture properties. Ultrasonic phase velocities and attenuation coefficients did not show significant differences between control and GOx noodles either before or after

72 h of refrigeration. However, refrigerated storage of control and GOx noodles did result in a significant increase in wave velocity and storage modulus ( $M'$ ) as well as a decrease in attenuation and  $\tan\delta_L$  (ratio of longitudinal loss modulus to longitudinal storage modulus), indicating increased firmness of noodle structure with storage time. Stress relaxation results on fresh unrefrigerated noodles showed an increase in Peleg's  $K_1$  and  $K_2$  parameters with GOx addition but did not resolve any significant changes in these parameters after 72 h of storage. This small amount of GOx did not improve cooked noodle texture, although noodle matrix changes during storage were clearly revealed by the noninvasive ultrasonic data.

Noodles, a traditional staple of the Asian diet, are a critically important food product for both the consumer and wheat exporting nations, with ~161 million metric tons of wheat used for noodle production annually (Bui and Small 2007). Yellow alkaline noodles are the dominant class of noodle, although their formulation and physical characteristics differ significantly to address local consumer preferences. Noodle manufacturers control the type of flour, protein content, and processing conditions because all have a significant impact on the final end product (Miskelly and Moss 1985; Kruger et al. 1994; Edwards et al. 1996; Hatcher et al. 1999, 2008; Hatcher and Anderson 2007).

Consumers in Japan, Malaysia, and Singapore have shown an increasing trend toward purchasing refrigerated noodles at the local convenience store during their hectic work day. An increasingly popular noodle in Japan is the Tsukemen noodle or “dipping” noodle. It is an alkaline ramen-type noodle, although unlike traditional ramen, it is cooked separately from the soup, chilled by washing with ice water, and served cold separately from the soup. Consumers appreciate its texture and its ability to take up the soup's flavor when dipped into either hot or cold soups. Most alkaline noodles are served in and cooked with the soup. Noodle manufacturers are aware that their consumers seldom eat the noodle and soup initially because it is too hot. Ensuring that the noodle retains a desirable texture after prolonged exposure to heat in a hot soup is therefore highly desirable.

Traditionally, higher flour protein content, in combination with a balanced gluten strength (maximum resistance to extension relative to extensibility via extensigraph), extensibility, and elasticity via gliadin and glutenin components, is necessary for a high-quality noodle product that offers the consumer a desirable noodle texture, combining a firmer bite with a chewy mouth-feel (Hatcher 2001). Glucose oxidase (GOx), initially used in the breadmaking industry

as an alternative to chemical oxidizers such as bromate, catalyzes the conversion of  $\beta$ -D-glucose into D-gluconic acid and additionally produces hydrogen peroxide. The advantage of using an enzyme, versus a chemical, to improve a product's texture is that it readily appeals to health conscious consumers who perceive enzymes as a natural and safe alternative to chemicals (Rosell et al. 2003; Bonet et al. 2006; Joye et al. 2009; Steffolani et al. 2010). It is the liberated hydrogen peroxide that causes formation of disulfide bonds (Rasiah et al. 2005) or crosslinks tyrosine to form dityrosine linkages (Tilley et al. 2001). These cross-linkages yield stronger protein networks with increased stability during mixing (Bonet et al. 2006).

Previous research with transglutaminase has demonstrated protein crosslinking in bread dough, primarily influencing the high-molecular-weight gluten subunits (Rosell et al. 2003; Autio et al. 2005), with a resulting decrease in dough extensibility (Rosell et al. 2003). Use of transglutaminase on noodles at the 2% w/w level by Bellido and Hatcher (2011) demonstrated a significant increase in both longitudinal storage modulus ( $M'$ ) and  $\tan\delta_L$  (ratio of longitudinal loss modulus to longitudinal storage modulus) values of the noodles, suggesting a desirable, much firmer noodle. GOx, however, is normally used at a much lower level (0.001–0.015% w/w) and offers a viable alternative. An additional major concern for refrigerated convenience noodle manufacturers is that the noodle may deteriorate upon storage owing to water loss. GOx has been observed to increase water absorption in doughs (Dagdelen and Gocmen 2007), possibly owing to the formation of an arabinoxylan gel (Vemulapalli et al. 1998; Miller and Hoseney 1999), offering the potential to overcome water loss issues.

Our lab has conducted extensive research into factors influencing the texture of Asian noodles. For example, previous research (Hatcher et al. 2009b) compared refrigerated yellow alkaline noodles made with Canada Western Amber Durum (CWAD) versus Canada Western Red Spring (CWRS). We showed that the CWAD noodles offered better texture parameters: maximum cutting stress, recovery, and resistance to compression when cooked after refrigerated storage for up to seven days.

One means of measuring the mechanical properties of noodles that relate to their texture is by use of ultrasonic techniques. Longitudinal ultrasound in a solid or viscoelastic material is an elastic wave at frequencies above 20 kHz, in which the material being tested is alternatively compressed and rarefied in the direction that the wave is traveling. It is well known (e.g., see McClements 1995) that the molecular bonds of a material's constituents influence how fast an ultrasonic wave propagates through the material, and the

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wave's attenuation depends on the strength, character, and dynamics of these molecular bonds. Bellido and Hatcher (2010, 2011) demonstrated that measurements of the sound velocity and attenuation could be used to discern differences in raw noodle rheological properties using an approach proposed by Elmehdi et al. (2004). Diep et al. (2014) confirmed this technique's usefulness by discriminating raw noodle rheological parameters as a function of wheat class and variety. Although effective, these papers utilized 40 kHz ultrasonic waves, requiring multiple layers of raw noodles to investigate differences in the noodles' rheological parameters. An alternative ultrasonic technique, for which a single layer suffices, is to use ultrasound of a higher frequency and to directly probe the matrix properties of the noodle dough, where the effect of enzymes such as GOx will be manifested.

The objective of this study is to use ultrasound in the 10 MHz range to discern and quantify differences in the rheological properties of cooked yellow alkaline noodle texture prepared with and without GOx in the formulation. Additionally, to address the needs of the refrigerated convenience noodle market, we examined the influence of GOx treatment on the cooked noodle's texture after the raw noodle had been refrigerated for three days prior to cooking.

## MATERIALS AND METHODS

**Wheat Samples, Milling, and Noodle Preparation.** The wheat represented a composite of CWRS samples collected across western Canada during the 2011 harvest and was milled to a 72% flour extraction level with an Allis-Chalmers mill. Flour (50 g), 34% water (w/w), 1% (w/w) NaCl, and 1% w/w kansui (9:1 w/w sodium and potassium carbonate) dissolved in the water, with or without 75 U of GOx (Sigma-Aldrich, Saint Louis, MO, U.S.A.), were mixed with a centrifuge mixer (SpeedMixer DAC 150FV, FlackTek, Landrum, SC, U.S.A.) for 30 s at 3,000 rpm (Diep et al. 2014). The aggregated dough crumbs were sheeted with an Ohtake laboratory noodle machine (Ohtake, Tokyo, Japan), undergoing a lamination step followed by seven passes according to the method of Bellido and Hatcher (2010) to duplicate commercial practices used to develop the gluten network within the dough sheet. The dough sheet was allowed to rest for 1 h in a sealed plastic bag to duplicate commercial manufacturing practices. Dough discs were cut from the dough sheet (41 mm diameter) with an aluminum circular cutter. Optimum cook time (8.0 min) was determined according to the method of Hatcher et al. (2009a). Upon completion of cooking, the discs were removed from the boiling water and immersed in room temperature water (22°C) for 2 min to stabilize. After the noodles were withdrawn, the excess surface water was removed by gentle contact with absorbent paper towels before placing the samples in a sealed plastic container. Any additional water was removed by gently blotting the disc against Kimwipes (Kimberly Clark, Irving, TX, U.S.A.) prior to placement between the polycarbonate plates attached to the transducers. Two cooked discs per dough sheet were analyzed from a minimum of three separate dough sheets. A second set of measurements was performed in the same way on noodle disks that were stored for 72 h at 4°C prior to cooking.

**Ultrasonic Measurements.** Ultrasound measurements were performed with two broadband 10 MHz Panametrics transducers (Olympus NDT Canada, Edmonton, AB, Canada), which were mounted on a TA.XTPlus texture analyzer (Texture Technologies, Scarsdale, NY, U.S.A.). The vertical position of the top transducer was controlled precisely by TA.XT Plus software, whereas the bottom transducer was fixed to the stationary base, allowing compression and relaxation tests to be performed and simultaneously monitored with ultrasound. Two polycarbonate plates (1/4 in. thick) were bonded to the transducer surfaces to provide a suitable time delay to the acoustic signals.

A cooked noodle sample was placed on the bottom delay plate, and the compression test was then initiated by gradually lowering the top transducer delay plate toward the noodle. When the top delay plate was about 3 mm above the noodle surface, the ultrasonic acquisition program (written in MATLAB [MathWorks, Natick, MA, U.S.A.]) was started, so that both the transmitted ultrasonic signals and the applied force could be monitored as soon as the top transducer delay plate touched the noodle. Distance measurements were calibrated as recommended by the equipment manufacturer, using a crosshead speed of 20 mm/s with a resolution of 0.01 mm (Diep et al. 2014). The cooked noodle disc thicknesses were determined based upon the calibrated starting height of the top transducer minus the distance traveled until achieving a contact force of 39 N with the noodle layer (Diep et al. 2014). An additional 20% strain was then imposed on the noodle disc and maintained through the 150 s analysis.

The ultrasonic measurements were initiated when the bottom transducer received a voltage signal from an arbitrary waveform generator (model 645, Berkeley Nucleonics Corp., San Rafael, CA, U.S.A.), generating an ultrasonic pulse that propagated through the noodle to the top transducer. Prior to each set of experiments, a reference pulse was recorded without the noodle disc, with a thin layer of ultrasonic coupling gel inserted between the delay plates to ensure good transmission. The received signal was amplified (Panametrics 5072 PR, Olympus), displayed on a digital oscilloscope (TDS 2024, Tektronix Canada, Toronto, ON, Canada), and captured by software developed in-house using MATLAB. The signals were averaged over 128 acquisitions to improve the signal-to-noise ratio. The digitized waveforms were analyzed with custom software developed in-house using IGOR 6 (WaveMetrics, Portland, OR, U.S.A.) to determine the phase velocity and attenuation. The attenuation coefficient and phase velocity were determined by comparing the magnitudes and phases of the fast Fourier transforms of the signal transmitted through the noodle disc relative to the signal obtained without it (e.g., see Strybulevych et al. 2007). Data at a frequency of 11 MHz, for which the signal amplitudes were the largest, were selected for comparison between the different noodle samples. These data, in conjunction with the measured cooked noodle density, were used to calculate the complex longitudinal modulus (Elmehdi et al. 2004). Cooked noodle density was determined by using the water displacement method according to Bellido and Hatcher (2010) right after the ultrasonic and relaxation tests.

**Stress Relaxation Measurements.** Stress relaxation measurements were performed concurrently on the cooked dough disc according to the method of Hatcher et al. (2008) with the TA.XTPlus texture analyzer. The software began collecting force measurements, 200 data points per second, once the 39 N force was achieved. The initial rate of relaxation ( $K_1$ ) and extent of relaxation ( $K_2$ ) were determined by the method of Peleg (1979), with transformation of the force versus time measurements calculated by using the macro provided with the TA.XTPlus unit. Relaxation and ultrasonic tests were performed concurrently on the same cooked noodle disc.

## RESULTS AND DISCUSSION

The inclusion of GOx into the noodle dough formulation increased the optimum cooking time of the fresh noodles by a small but measurably longer period, from 8 to 8.5 min as determined by the loss of the visible core during the squeeze test. A minor influence of GOx (and the associated difference in cooking time) on the attenuation is seen in Table I. The fresh unrefrigerated GOx sample did not display any difference in wave velocity relative to the corresponding control sample. Similar behavior was seen for refrigerated noodles, with the velocity of GOx-treated samples not being significantly different from the control ones. Although the addition of GOx to either fresh or refrigerated

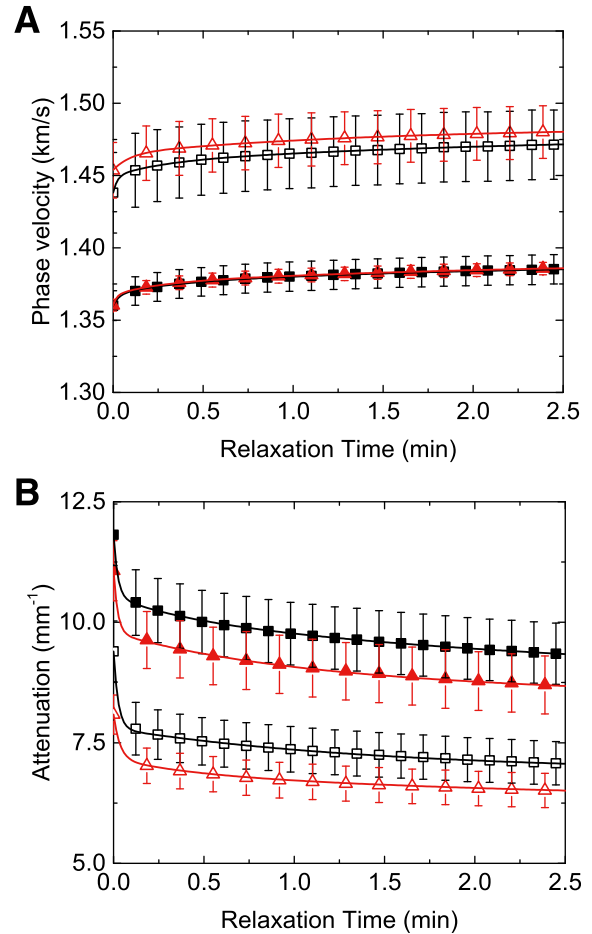
noodles appeared to cause a slight reduction in attenuation, this decrease was not significant. However, significant differences in both the velocity and attenuation are seen in Table I as a result of refrigerated storage for 72 h prior to cooking, with the velocities of both control and GOx noodles showing an increase with storage time and the attenuation showing a decrease. These differences are also clearly seen in Figure 1, in which the relaxation time dependence of the phase velocity (Fig. 1A) and attenuation (Fig. 1B) are plotted.

Both Figures 1A and 1B highlight the observations that neither the phase velocity nor the attenuation are significantly affected by the addition of GOx. This observation is somewhat surprising in that Bonet et al. (2006) in their study of the influence of GOx on bread dough microstructure found that GOx treatment, at extremely low levels (0.001% w/w), resulted in an increased number of pores of sizes that would be anticipated to increase the attenuation coefficient of our noodles. They reported that increasing the GOx levels 10-fold resulted in a more discontinuous protein matrix structure, so that the matrix's original orientation was completely lost. The lack of a similar effect on noodles may be attributable to their much lower water absorption level, the lack of fermentation, and less intense dough development. Previous research has been primarily directed toward studying the effect of GOx addition on breadmaking performance by using standard farinograph water absorption levels, which involve ~55–73% water addition (Vemulapalli et al. 1998; Primo-Martín et al. 2003) and which are significantly higher than those used in the preparation of noodles (34–37%). Also, a significant difference in the effect of GOx on bread dough and alkaline noodle dough structure might be owing to the alkaline environment of noodles. Alkaline salts shift the pH to higher values, for which GOx activity is reduced up to 40% (Hashemifard et al. 2010). Our kansui salt solution pH is 11, whereas maximum GOx activity as an enzyme is observed at neutral pH environments.

The longitudinal storage modulus ( $M'$ ) reflects how well the cooked noodle stores the acoustic strain energy of the ultrasonic pulses (Bellido and Hatcher 2010). Equivalently, the magnitude of  $M'$  determines the magnitude of the applied stress needed to achieve a particular longitudinal (elastic) deformation, so that a larger value of  $M'$  implies a firmer (less compressible) material. Calculated  $M'$  values from the measured velocity, attenuation, and density are shown in Figure 2A. Addition of the GOx at this low level, 0.0009%, had no significant impact on the noodle's  $M'$  values for either the fresh or the refrigerated noodles. However, both control and GOx noodles displayed significantly greater  $M'$  values than the fresh noodles following 72 h of refrigeration at 4°C. There was some evidence (taken from across the whole relaxation time course) that  $M'$  was larger in GOx-treated noodles, indicative of a firming taking place in the enzyme-treated noodles during 72 h of refrigerated storage. In wheat products such as noodles, starch is the most abundant component, and when cooked, starch granules gelatinize and lose their crystalline structure. Starch polymers are released from the protein network as the granule swells (Ross et al. 1997; Hatcher et al. 2009b). Accordingly, reagents such as alkaline

salts, which affect the starch and proteins (by increasing disulfide linkages and decreasing free sulfhydryl groups) during refrigerated storage (Shiau and Yeh 2004; Hatcher et al. 2008), will alter protein denaturation during cooking as well as protein network disruption by the starch granule swelling. As a result, the cooked noodle will have a tighter, firmer noodle texture and less cooking loss, as is seen in the changes in the ultrasonic velocity and attenuation and the corresponding increase of  $M'$ .

The extent to which acoustic energy is dissipated in the cooked noodles is indicated by the loss modulus ( $M''$ ), which is shown in Figure 2B. In light of the findings on  $M'$ , it was interesting to note that the addition of GOx to the formulation did not significantly alter the  $M''$  of the enzyme-treated cooked



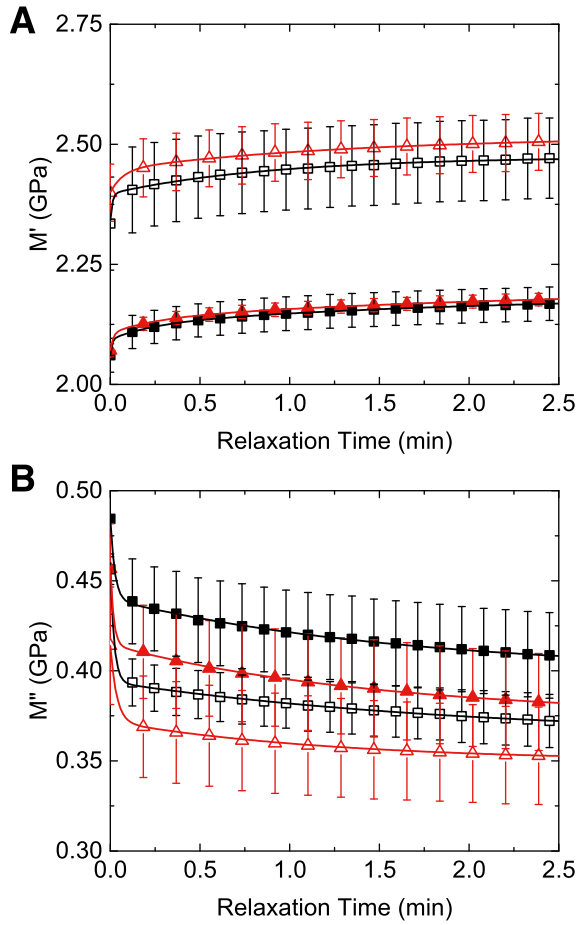
**Fig. 1.** Phase velocity (A) and attenuation coefficient (B) at 11 MHz as a function of relaxation time for cooked noodles made from Canada Western Red Spring flour (control) after 0 h (■) and 72 h (□) and with the addition of 75 U of glucose oxidase (9 ppm) after 0 h (▲) and 72 h (△).

**TABLE I**  
Influence of Glucose Oxidase (GOx) Addition on the Phase Velocity and Attenuation (at 11 MHz) for Cooked CWRS Wheat Flour Noodle Discs After 0 and 72 h of Refrigeration<sup>a</sup>

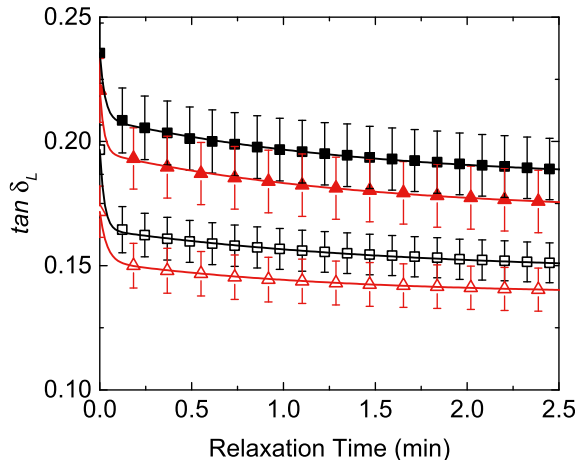
Ultrasonic Parameter	After 0 h of Refrigeration				After 72 h of Refrigeration			
	Control (CWRS)		GOx Addition		Control (CWRS)		GOx Addition	
	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%
Phase velocity (km/s)	1.38 ± 0.01	0.73	1.38 ± 0.005	0.35	1.46 ± 0.02	1.67	1.47 ± 0.02	1.25
Attenuation coefficient (mm <sup>-1</sup> )	9.94 ± 0.66	6.64	9.27 ± 0.60	6.46	7.48 ± 0.49	6.49	6.82 ± 0.36	5.32

<sup>a</sup> Uncertainties of the means are the standard errors,  $SD/(n^{0.5})$ , where SD is standard deviation and  $n$  = number of replications (minimum of three). Values of velocity and attenuation are reported after 1 min of compressive stress relaxation. CWRS = Canada Western Red Spring, and CV% = coefficient of variation %.

noodles relative to the control. Storing the noodles at 4°C for 72 h, although reducing the  $M''$  values for both control and GOx-treated noodles, did not exert a statistically significant effect. Analysis of  $\tan\delta_L = M''/M'$  (Fig. 3) shows a decrease in  $\tan\delta_L$  for both control and GOx noodle dough, indicating a firming of the



**Fig. 2.** Relaxation time dependence of  $M'$  (A) and  $M''$  (B) at 11 MHz for noodles made from Canada Western Red Spring flour (control) after 0 h (■) and 72 h (□) and with the addition of 75 U of glucose oxidase (9 ppm) after 0 h (▲) and 72 h (△).



**Fig. 3.** Comparison of  $\tan\delta_L$  values ( $\tan\delta_L = M''/M'$ ) of the cooked noodle sheet prepared with Canada Western Red Spring flour (control) after 0 h (■) and 72 h (□) and with the addition of 75 U of glucose oxidase (9 ppm) after 0 h (▲) and 72 h (△).

texture with refrigeration time. However, a significant influence of GOx addition on  $\tan\delta_L$  at the same observation times is not evident.

Peleg's stress relaxation parameters were calculated based upon the uniaxial stress relaxation curves (Table II). Higher  $K_1$  and  $K_2$  values are associated with firmer food materials (Singh et al. 2006). With the exception of a comparison of the refrigerated GOx-treated noodle and its control counterpart for the  $K_2$  parameter, the stress relaxation measurements indicated two trends in enzyme treatment and the effect of storage. First, the stress relaxation parameters were larger for the GOx treatment compared with the control. Second, the refrigerated samples had larger values compared with the fresh samples. However, these differences were not significant at the 5% level. The trends in stress relaxation parameters with treatment were consistent with the rheological determinations of cooked noodle texture from ultrasound with the exception of storage effects, for which the relaxation parameters were not found to be as sensitive to the significant increase in texture firmness with storage time that was detected in the ultrasonic  $M'$  data. Nonetheless, the trends noted for stress relaxation were mostly observed for the  $\tan\delta_L$  values in Figure 3, for which the trends to lower  $\tan\delta_L$  values matched well with the larger  $K_1$  and  $K_2$  values that are associated with firmer food materials (Singh et al. 2006). Lower  $\tan\delta_L$  values correspond to smaller relative losses of the acoustic energy as the pulses propagate through the cooked noodles, behavior that is expected in firmer noodles. Thus, for this examination of enzyme treatment and refrigerated storage, small and large strain measurements generally corresponded quite well with each other. However, our ultrasonic and stress relaxation data did not conclusively show that there is a positive improvement in cooked noodle firmness as a result of the addition of small quantities of GOx, although there were encouraging trends in the data suggesting that GOx addition may still have merit for satisfying consumer preference for firmer convenience-store cooked noodles.

## CONCLUSIONS

This is the first application of which we are aware that demonstrates the use of ultrasonic measurements to study cooked noodle rheological properties. The ability to perform measurements on single noodle sheets offers significant advantages in time and material over our previous investigations employing multiple layers of raw noodles (Bellido and Hatcher 2010, 2011; Diep et al. 2014; Hatcher et al. 2014). The use of the higher frequency, 11 MHz, allows the study of the underlying matrix structure without complications from microbubbles embedded in the cooked noodle. Clear effects of refrigerated storage for 72 h were found on the mechanical properties of the noodles as assessed ultrasonically. An influence of low levels of GOx addition to the formula was not observed. The determination of the traditional Peleg's  $K_1$  and  $K_2$  values from simultaneously

**TABLE II**  
Peleg's  $K_1$  and  $K_2$  Values Derived from the Linearization of the Stress Relaxation Curves Simultaneously Measured with the Ultrasonic Assembly<sup>a</sup>

Sample	$K_1$	$K_2$
Control 0 h	2.79 ± 0.10	6.66 ± 1.00
GOx-treated 0 h	2.95 ± 0.052	7.76 ± 0.40
Control 72 h	2.96 ± 0.09	7.51 ± 0.64
GOx-treated 72 h	3.09 ± 0.08	7.51 ± 0.57

<sup>a</sup> Uncertainties are the standard error,  $SD/(n^{0.5})$ , where SD is the standard deviation and  $n$  is number of replications (minimum of three).  $K_1$  represents the initial rate of relaxation;  $K_2$  represents the extent of relaxation; and GOx = glucose oxidase.

conducted stress relaxation measurements provided information on the rheological properties that was complementary to the ultrasonic results.

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