



The effect of mixing conditions on the material properties of an agar gel—microstructural and macrostructural considerations

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Abstract

The effect of mixing on the properties of agar gels was investigated with consideration being given to both macrostructural and microstructural characteristics of the gel through rheological techniques that include conventional and ultrasound based methods and Differential Scanning Calorimetry.

Agar gels of 1 and 3% concentrations were prepared. The gels were subjected to three different mixing conditions: no mix, slow mix (400 rpm), and fast mix (900 rpm). A pulse echo ultrasound technique was utilized to obtain velocity measurements and these velocity measurements were used to obtain ultrasound derived mechanical modulus values. A controlled-stress rheometer was used to obtain rheological properties of the gel as well. Differential Scanning Calorimetry (DSC) was performed to determine thermal transitions of the various systems studied in order to obtain information on the degree of microstructural association of the agarose fractions.

Mixing speed affected the degree of porosity induced in the system, by incorporation of the air bubbles, and the rheology of the system. Both ultrasound derived mechanical properties and those obtained in a conventional rheometer showed that mixed gels were stronger than the no mix gels in spite of the no mix gels exhibited negligible porosity, i.e. they did not have bubbles that could weak the macrostructure of the gels. In addition, DSC results indicated that gels mixed at different conditions exhibited thermal transitions at different temperatures. These results showed that material properties of agar gels might be more sensitive to changes in the microstructure than the macrostructure of these systems. This highlights the importance on preparation conditions and utility of an agar gel.

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Keywords: Mixing; Agar gels; Viscoelasticity; Ultrasound

1. Introduction

Agar is a gel forming polysaccharide with a main chain consisting of alternating 1,3-linked β -D-galactopyranose and 1,4-linked 3,6 anhydro- α -L-galactopyranose units (Arnott, Fulmer, & Scott, 1974). Agarobiose is the basic

disaccharide structural unit of all agar polysaccharides. Agar can be fractionated into two components: agarose and agaropectin (Labropoulos, Niesz, Danforth, & Kevrekidis, 2002). Agarose is a neutral polysaccharide and it is the fraction with the greatest gelling capacity. The other fraction of agar, agaropectin, contains the charged polysaccharide components. In agaropectin some residues are replaced with a pyruvic acid ketal, 4,6-O-(1-carboxyethylidene)-D-galactopyranose or by methylated or sulphated sugar units (Labropoulos et al., 2002). Also, the agarose and agaropectin contents vary and are depending on the seaweed source from which the agar was extracted. These facts are of importance as they will affect the physicochemical, mechanical, and rheological properties of agar (Labropoulos et al., 2002).

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113 Djabourov, Clark, Rowlands, and Ross-Murphy
114 (1989) indicated that there is no universal explanation for
115 the gelation mechanism of agar gels, however, they noted
116 that studies have been performed which suggest that the
117 transition from an agarose coil to a double helix is the origin
118 of the gelation process. It has been suggested that the
119 agarose double helix network, which forms the gel, can be
120 described as arising by both double helix formation and by
121 subsequent aggregation of these helices into bundles, called
122 suprahelices (Djabourov et al., 1989; Labropoulos et al.,
123 2002).

124 Agar gels have a number of practical applications.
125 In laboratory work, agar gels act as bacterial culture
126 supports, separation media in column chromatography and
127 electrophoresis (Djabourov et al., 1989) and therefore their
128 properties must be maintained within very narrow specifica-
129 tions. Agar gels have found use in medicine and pharmacy
130 (Watase, Nishinari, Clark, & Ross-Murphy, 1989). In the
131 food industry, agar gels are commonly used as thickening
132 and gelling agents (Djabourov et al., 1989; Watase et al.,
133 1989). The physicochemical, mechanical, and rheological
134 properties of agar gels are extremely important. It is also
135 important to be aware of factors that may affect the
136 properties of agar gels. A key reason for the function of
137 agar gels is that they have high elastic moduli at low
138 concentrations (Djabourov et al., 1989). Work has been
139 performed by many authors, which cite the direct relation-
140 ship between gel concentration and the strength of the gel
141 (Clark et al., 1988; Djabourov et al., 1989; Moritaka,
142 Nishinari, & Horiuchi, 1980; Watase et al., 1989). Agar
143 forms a thermoreversible gel in aqueous solution and the gel
144 remains stable over a wide temperature range; agar gels
145 have a gelling/setting temperature close to 40 °C and a
146 melting temperature near 90 °C (Djabourov et al., 1989;
147 Moritaka et al., 1980). Literature indicates a
148 direct relationship between gel concentration and gel
149 melting temperature (Eldridge & Ferry, 1954; Moritaka
150 et al., 1980).

151 These statements are not novel and have long been
152 accepted in the literature concerning the properties of
153 agar and other polysaccharide-derived gels. However,
154 more recent work from Inoue et al. (2002) on alginate
155 based materials used in clinical dentistry indicated that
156 there is a noticeable effect of the mixing method on the
157 rheological properties of the unset alginate pastes and
158 the set alginate materials. Their work indicated that when
159 the alginate pastes were mixed with high-speed rotary
160 mixers, the set alginate materials exhibited greater gel
161 strength than the set alginate materials prepared with a
162 slow speed hand-mixing technique. Inoue et al. (2002)
163 attributed this result to the fact that the high-speed rotary
164 mixed set alginate materials may contain less air bubbles
165 than the set alginate materials prepared with a hand-
166 mixing technique. Inoue et al. (2002) proposed that this
167 seems to indicate that air bubbles are removed during
168 fast speed mixing as a result of the difference in density

of the paste and the air as the paste is rotated at high 169
speeds. However, it was also noted by Inoue et al. (2002) 170
that there are many ambiguous properties regarding the 171
effect of mixing on alginate pastes (unset alginate 172
materials) and set alginate materials. Thus, a discussion 173
on the concepts of mixing and air bubbles with regard to 174
well-studied systems is warranted. 175

The effect of mixing on the presence of air bubbles 176
has been well studied in the field of dough rheology 177
(Campbell, Rielly, Fryer, & Sadd, 1998). In fact, one of 178
the most important ingredients present in dough-based 179
products is air. Air arises from air entrapped in the bulk 180
volume of the flour mass or from entrainment during the 181
mixing process (Scanlon & Zghal, 2001). One aspect of 182
the increase in the air volume fraction of dough during 183
mixing that has been studied was to assess whether 184
mixing promotes an increase of the size of the original 185
air bubbles or an increase in the number of bubbles of 186
the same size. Based on the work of Campbell et al. 187
(1998) where dough was mixed under a vacuum 188
(i.e. little air), it was noted that a fewer number of air 189
bubbles were entrained rather than smaller sizes of the 190
same number of bubbles. From this, it appears that 191
mixing increases the number of bubbles rather than 192
increasing the size of the bubbles present in the system 193
(Scanlon & Zghal, 2001). This is important as ultimately, 194
these differences in the number and size of air bubbles 195
are expected to give rise to different macrostructures, i.e. 196
different pore sizes and porosities due to the different 197
number and size of air bubbles (Campbell et al., 1998; 198
Scanlon & Zghal, 2001). Literature has indicated that the 199
degree of porosity, which provides the macrostructure of 200
a material, affects the mechanical properties of the 201
material (Attenburrow, Goodbrand, Taylor, & Liliford, 202
1989; Gibson, Ashby, Schajer, & Robertson, 1982). 203

204 Furthermore, mixing speed affects the bubble size
205 present in mixed systems. Hanselmann and Windhab
206 (1999) studied the effect of mixing speed and air bubble
207 size in whey protein isolate foams. They indicated that, as
208 mixing speed increased, the flow field around the mixer
209 blades transitioned from laminar to turbulent. They also
210 noted that a turbulent flow field was preferred in terms
211 of gas dispersion thereby promoting the break up of larger
212 bubbles into a larger number of smaller bubbles
213 (i.e. increasing the number of air bubbles).

214 The overall objective of this work was to determine the
215 effect of mixing on material properties of an agar gel with
216 consideration being given to both macrostructural and
217 microstructural aspects. The specific objectives of this
218 research were: (1) to study the effect of mixing speed on
219 bubble formation in an agar gel; (2) to use ultrasound and
220 rheological measurements to characterize the effect of
221 different bubble sizes on material properties, and (3) to use
222 Differential Scanning Calorimetry (DSC) to investigate
223 possible shifts in key thermal transitions and how these
224 shifts are affected by mixing.

2. Methods and materials

2.1. Agar gel preparation

Agar gels of concentrations 1 and 3% (w/v) were prepared by dissolving granulated agar (Sigma, St Louis, MO) in distilled water. Each solution was heated over a hot plate in a Pyrex beaker until a temperature of 98 °C was attained. The agar dispersion was placed in an ice bath to rapidly cool down the dispersion until a temperature of 40 °C was reached. After that the dispersion was cooled down to below 40 °C, and poured into 1000 ml square Ziploc disposable storage containers. Once the dispersions were transferred to these containers, they were subjected to one of two different treatments. *Treatment 1*: it involved letting the gels set without any mixing (i.e. no bubbles were present in the gels). *Treatment 2*: it involved mixing the gels with a household handheld mixer for 90 s. Two mixing settings were used and the rpm values were determined, by a tachometer, as 400 and 900 rpm for the low and high speeds, respectively. The different mixing speeds were employed in order to incorporate different sized bubbles into the gels. Based on the work by Hanselmann and Windhab (1999) it was assumed that different mixing conditions would create gels with different bubble size distributions and therefore different degrees of porosity. The gels were allowed to set for 4 h at refrigeration conditions (i.e. 7 °C).

2.2. Ultrasound work

2.2.1. Experimental set-up

The experimental set-up consisted of a pulse generator-receiver (model 5800, Panametrics, Waltham, MA), a 3.5 MHz piezoelectric transducer (V609, V155, Panametrics, Waltham, MA), a delay line (Panametrics, Waltham, MA), a custom-made measurement cell, and a PC with acquisition hardware and data analysis software (LABVIEW for Windows, National Instruments, Austin, TX). A silicone couplant was used between the delay line and the transducer to ensure good contact and transmission of ultrasound waves. The experimental set-up employed to measure the ultrasonic velocity and attenuation of the model food system (agar gel) was a pulse echo technique. All experiments were performed in triplicate.

2.2.2. Principles of experimental set-up

The principle of the pulse echo technique involves the utilization of a pulse generator-receiver. The pulse generator-receiver produces electrical signals that are converted into ultrasonic waves by the piezoelectric transducer. The ultrasonic waves (Fig. 1) travel along a delay line. When the ultrasound wave reaches the interface between the delay line and the sample, a portion of the energy is reflected at the boundary of the delay line and the sample and a portion of the energy is transmitted through the sample. The transmitted waves that propagate through the sample reach

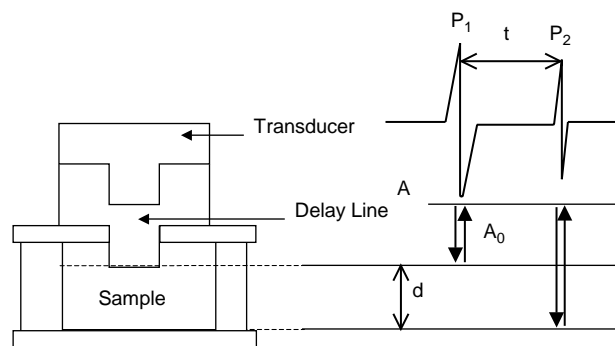


Fig. 1. Schematic of ultrasound set-up.

the boundary between the sample and the measurement cell where they are reflected back to the transducer. The transducer receives the reflected waves that have traveled back to the transducer. These reflected waves are converted back into electrical signals that are recorded in a computer. The effect of mixing and the presence of bubbles on the properties of the agar gels were determined from measurements of ultrasonic velocity.

2.2.3. Analysis of experimental results

The ultrasonic velocity and attenuation coefficient of the sample were each determined by analyzing the reflected signals, which are designated P_1 and P_2 in Fig. 1. Ultrasonic velocity was determined from the thickness of the sample, d , and the time difference, t , between the signal (P_1) reflected from the interface of the delay line and the sample (i.e. the front face of the sample) and the signal (P_2) reflected from the interface of the sample and the measurement cell (i.e. the back face of the sample). This time difference, t , represents the two-way travel path through the sample. Therefore, the longitudinal velocity of the ultrasound waves through the sample was determined by Eq. (1)

$$V = \frac{2d}{t} \quad (1)$$

For a compressional wave the ultrasonic velocity V is related to the square root of the material elastic modulus E and its density ρ (Povey & McClements, 1988). The relationship is given by the following equation:

$$V = \sqrt{\frac{E}{\rho}} \quad (2)$$

A compressional wave can propagate in both liquids and solids and in general ultrasound waves can propagate through a solid material faster than through a liquid material because a solid material resists shearing. A shear wave (transverse wave) can propagate through most solid materials, yet in general, the shear velocity is less than that of a longitudinal wave. Also, shear waves are generally more attenuated than longitudinal waves. Other experiments were performed using shear transducers with the aim of determining the shear velocity of the model food system

(agar gel). The signal was highly attenuated. In an attempt to circumvent this problem very thin slices of sample were placed under the transducer. The thin sample was observed to deform under the load of the transducer. Therefore, shear velocity measurements of the agar gel were not determined and Eq. (2) was used to obtain elastic modulus values from longitudinal velocity measurements.

2.3. Low frequency rheological measurements

A controlled stress rheometer (Viscotech, Lund, Sweden) was used to perform both strain and frequency sweep experiments on the 1 and 3% agar gels. A serrated plate–plate geometry was used with a 2 mm gap. The serrated plates were chosen in order to minimize slippage. Both strain and frequency sweep tests were performed at room temperature (25 ± 2 °C). Dynamic strain sweeps for both the 1 and 3% agar gels were performed at a frequency of 1 Hz in order to determine the strain level in the linear elastic regime. At a strain of 0.05 the gels were within the linear viscoelastic regime. The strain controlled dynamic frequency sweep for the 3% agar gel employed frequencies ranging from 0.1 to 10 Hz and a strain level of 0.05. The strain controlled dynamic frequency sweep for the 1% agar gel employed frequencies ranging from 0.01 to 2 Hz and a strain level of 0.05. It should be noted that a lower frequency range was adopted for testing the 1% agar gel. This was due to the fact that slippage was observed at higher frequencies for these samples. A cylindrical 2 cm diameter cork borer was used to obtain samples for testing. Samples were cut to a 2.2 mm thickness. The purpose of the rheology tests was to determine the effect of different bubble sizes on material properties. All measurements were performed in triplicate. Also the rheological measurements were compared against the ultrasound velocity measurements.

2.4. Quasi-static compression tests

An Universal Testing Machine (Sintech MTS Model 10, Eden Prairie, MN) equipped with a 10 lb load cell, was used to perform uniaxial quasi-static compression tests on the agar gels that had been mixed under various mixing conditions. A crosshead speed of 1 mm/min was employed. All compression testing was performed at room temperature (25 ± 2 °C). A cork borer (2.0 cm diameter) was used to obtain cylindrical specimens of 2.0 cm height. The cylindrical specimens were compressed to failure with a normal force under lubricated platens and canola oil as the lubricant. The force–deformation data of the cylindrical specimens was used to calculate the elastic modulus. The elastic modulus is the proportionality constant that relates stress and strain (Beer & Johnson, 1992). Axial (i.e. tensile or compressive) stress (σ) is the applied force divided by the area perpendicular to the applied force (i.e. F/A). Strain (ϵ) is a measure of relative deformation caused by the stress or the change in length of the sample divided by the original

length of the sample (i.e. $\Delta L/L$). The elastic modulus is calculated by the following equation

$$E = \frac{\sigma}{\epsilon} \quad (3)$$

Experimentally, the elastic modulus was calculated from the slope of the stress–strain curve in the 1–4% strain region and from specimen dimensions. All trials were performed in triplicate.

2.5. Differential scanning calorimetry (DSC)

The agar gels prepared under various mixing conditions were tested with a DSC operated on standard scanning mode (2920 Modulated DSC, TA Instruments, New Castle, DE). The parameters used for Differential Scanning Calorimetry (DSC) were a temperature increase of 2 °C/min and a temperature range of 5–100 °C. The samples were contained in hermetically sealed aluminum pans. The amount of agar gel tested per sample was 7.21 ± 0.21 mg.

2.6. Microscopy

Microscopy was performed with a stereomicroscope (Nikon, Japan). For the samples that were subjected to different mixing speeds, 5 ml of liquid dispersion after a specified mixing treatment was dispensed as a 2 mm thin layer of a transparent Petri plate. An overhead transparency of graph paper was placed under the Petri plate to ensure that the same cross-sectional area was viewed under the microscope. For each mixing condition five micrographs were viewed. The bubbles on these micrographs were analyzed with an image analysis system (IPLAB 3.6, Scanalytics, Fairfax, VA).

2.7. Density and air fraction measurements

The density and air bubble fraction of the agar gels that were mixed under different conditions were measured. The use of the term air fraction refers to the amount of air in the samples as affected by the size and number of bubbles induced by mixing. The term air fraction is used instead of the term porosity as it is realized that gels have an inherent porosity regardless of whether inclusion of air bubbles upon mixing has occurred or not. It is by virtue of this inherent porosity that gel chromatography separation techniques are based. Therefore, the term air fraction has been employed to represent the air entrained (in the form of bubbles) in the gel samples upon mixing. All density determinations were performed at room temperature (25 ± 2 °C). A cork borer (2.0 cm diameter) was used to obtain cylindrical specimens of 2.0 cm height, which were used for measuring density. The mass of the samples was read on an electronic (± 0.01 g) balance (Denver Instruments Co., Denver, CO). The average of three readings was noted. The specimens had a simple cylindrical volume, which was calculated from

the dimensions of the cylindrical samples as $v = (\pi D^2/4)h$ where D and h are the diameter and the height of the sample. All density determinations were performed in triplicate. The density was obtained by the following equation

$$\rho = \frac{m}{v} \quad (4)$$

where m is the mass and v the volume of the sample.

Furthermore, the air bubble fraction (ϕ) is defined as the volume of air occupied by the bubbles in the sample fraction or alternatively the void fraction. The air fraction measurements were taken relative to the agar gel that received no mixing. It was assumed that the no mix gel was void of air bubbles (i.e. there was no air fraction) and the no mix gel can be considered the reference to which the other samples were compared against. Air bubble fraction was estimated from the following equation (Clayton & Huang, 1984)

$$\phi = 1 - \frac{\rho_b}{\rho_s} \quad (5)$$

where ρ_b is the density of the mixed gels, and ρ_s is the density of the no mix gel which was assumed as not having air bubbles.

3. Results and discussion

3.1. General relationship between mixing conditions and low frequency rheological measurements

The relationship between mixing speed and low frequency rheological measurements was investigated. This was achieved by preparing agar gels with both 1 and 3% concentrations and with different mixing conditions. Figs. 2 and 3 show the complex modulus, G^* , as a function of frequency, for gels of concentrations 1 and 3%, respectively. The figures clearly illustrate that for both concentrations, agar gels prepared with the fastest mixing speed had the largest modulus. Also, as expected stronger gels were produced at higher concentrations. In addition to the absolute value of the complex modulus, the elastic nature of the gels formed can be evaluated by examining changes of the complex modulus G^* with the frequency. As shown in Figs. 2 and 3, plots of G^* versus frequency in logarithmic coordinates are fairly linear. That enables the use of a power law model to describe changes in the modulus with the frequency. Power law models have been used to describe the rheological behavior of various gels systems (Chambon & Winter, 1987; Winter & Chambon, 1986) and has been demonstrated that for materials following a power law model small strain dynamic oscillatory measurements can be used to estimate the relaxation modulus of these materials using well defined analytical functions (Campanella & Peleg, 1987).

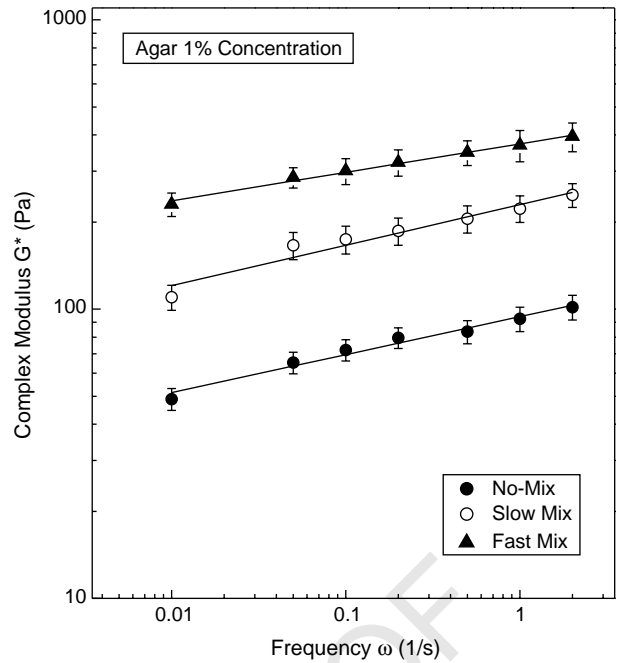


Fig. 2. The effect of mixing speed on the complex modulus for a 1% agar gel.

The power law model can be expressed as:

$$G^* = a\omega^n \quad (6)$$

The slope of these linear plots, given by the parameter n provides a good indication on the elastic nature of the gels in

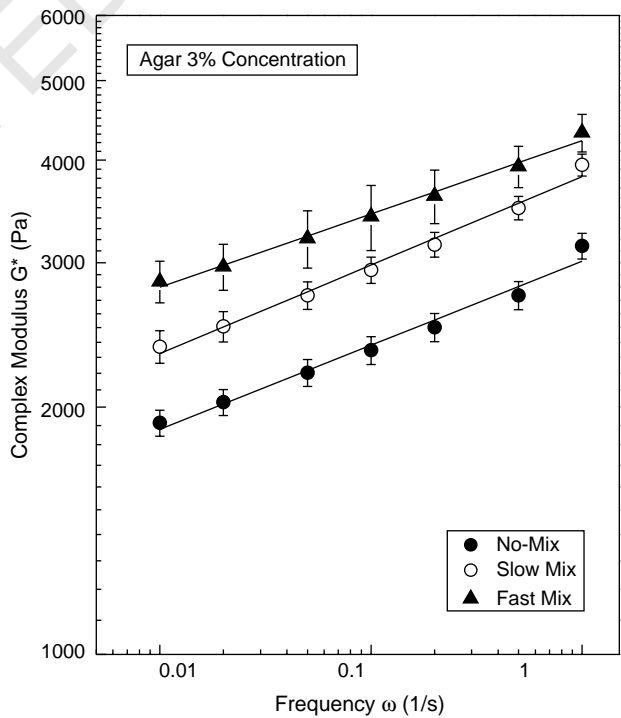


Fig. 3. The effect of mixing speed on the complex modulus G^* for a 3% agar gel.

561 addition to the magnitude of the modulus, defined by the
 562 parameter a in Eq. (6). It is known that the slope of these
 563 plots decrease when the material becomes more elastic,
 564 being equal to zero for pure elastic materials. Linear
 565 regression applied to the data showed that there was a
 566 significant decrease in the slope of the plots for fast mixed
 567 gels, whereas no differences were detected between no
 568 mixed and slow mixed gels. Results of concentration effects
 569 were expected because they have been commonly noted in
 570 the literature (Clark & Ross-Murphy, 1985; Hermans, 1965;
 571 Mitchell, 1976; Morris & Chilvers, 1983; Nussinovitch, Ak,
 572 Normand, & Peleg, 1990; Oakenfull, 1984), but the effect of
 573 mixing on the frequency response of agar gels has not been
 574 reported before.

575 Linear regression on the G^* versus ω plots showed that
 576 for non-mixed and slow mixed 1% concentration gels the
 577 slope were 0.13 and 0.14 and 0.10 for the fast mixed gel,
 578 respectively. In addition for 3% concentration gels the
 579 slopes were 0.10 for no mixed and slow mixed gels and 0.09
 580 for fast mixed gel. The small values of the slope for all the
 581 samples tested would be indicating that although these gels
 582 exhibit viscoelastic behavior from their frequency spectrum
 583 they could be assumed as approximately pure elastic
 584 material.

585
 586 *3.2. Relationship between mixing conditions and material*
 587 *properties as determined by ultrasound and compression*
 588 *tests—macrostructural considerations*
 589

590 Before discussing the results of uniaxial compression
 591 elastic modulus values and ultrasound derived elastic
 592 modulus values presented in Table 1, the conclusions
 593 obtained in the previous section in regard to the viscoelastic
 594 nature of the samples tested will be considered for the
 595 analysis of these results. Conventional small strain oscil-
 596 latory rheometry has shown that the samples could be
 597 considered as approximately elastic materials. It is noted
 598 that there may be questions to the validity of this assumption
 599 as gels can be considered to be viscoelastic materials, in that
 600 they possess both elastic and viscous components, but our
 601 results are indicating that the elastic component of the
 602 studied gels exceed by far their viscous components. Large
 603 values of the storage modulus values G' as compared to the
 604 measured loss modulus values G'' (data not shown) would
 605

617 be indicating the elastic nature of these gels. Furthermore,
 618 based on the work of Benedito, Carcel, Gonzalez, and
 619 Sanjuan (2000) it may be valid to consider a gel to be an
 620 elastic material and therefore measurement of the elastic
 621 modulus through uniaxial compression tests and longitudi-
 622 nal velocity measurements may be suitable. Generally, the
 623 bulk modulus of a gel is 2×10^9 Pa, while the shear modulus
 624 of a gel is 1000 Pa. The bulk modulus is nearly six orders of
 625 magnitude larger than the shear modulus. Based on these
 626 facts Benedito et al. (2000) noted that gels could be
 627 considered elastic materials and consequently any changes
 628 in ultrasound velocity would be mainly due to changes in
 629 the bulk or the elastic modulus of the material.

630 Table 1 gives results obtained from the ultrasound and
 631 uniaxial compression tests. It shows that for agar gels of 1
 632 and 3% concentrations there is an effect of mixing speed on
 633 ultrasonic velocity and air fraction/bubbles. Gels with the no
 634 mix treatment had the slowest ultrasonic compressional
 635 velocity while gels with the fast mix treatment had the
 636 highest ultrasonic compressional velocity. Based on
 637 ANOVA performed at $P=0.05$, there is only a significant
 638 difference between the ultrasonic velocity measurements for
 639 the no mix and fast mix conditions for both the 1 and 3%
 640 agar gel concentrations. These results are in agreement with
 641 the conventional rheology results described in the previous
 642 section. There are significant differences between the
 643 compressive elastic modulus for the no mix, slow mix,
 644 and fast mix conditions for both the 1% agar gels and 3%
 645 agar gels. These results are based on ANOVA performed at
 646 $P=0.05$.

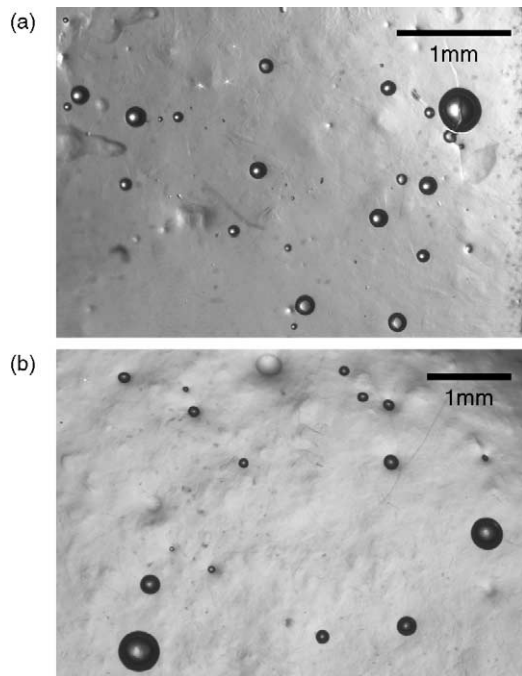
647 The fast mix gels had a lower air bubble fraction, while
 648 the slow mix gels showed the largest air bubble fraction.
 649 These results are in agreement with the results of Inoue et al.
 650 (2002) as they stated that set alginate materials prepared by
 651 hand-mixing, which was the slow mixing condition imposed
 652 by those researchers, contained more air bubbles than set
 653 alginate materials prepared by mechanical mixing, which
 654 was the fast mixing condition imposed by those researchers.
 655 The explanation proposed by Inoue et al. (2002) stated that
 656 more air bubbles are removed during fast speed mixing, as a
 657 result of the difference in density of the alginate paste and
 658 the air, as the paste is rotated at high speeds.

659 As already mentioned, the increase in air volume
 660 achieved by mixing is biased towards creating larger
 661

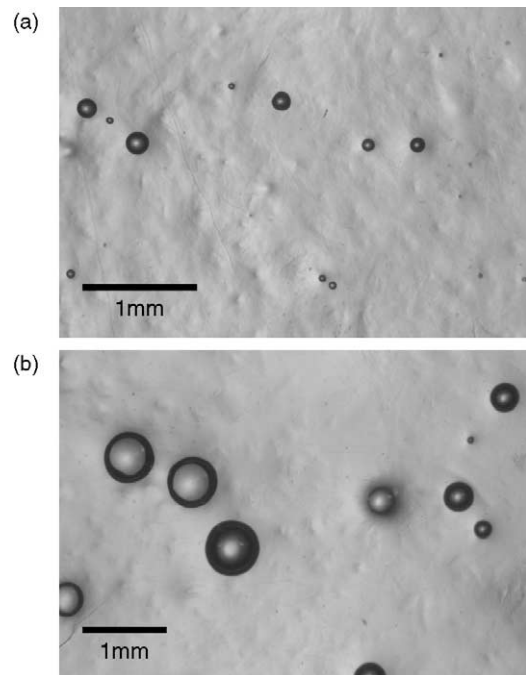
606 Table 1
 607 Comparison of ultrasound velocity, ultrasound derived elastic modulus, compressive elastic modulus, and air bubble fraction measurements with gel
 608 concentration and mixing speed

609 Gel conc. and mix	610 Velocity	611 Ultrasound derived	612 Compressive elastic	613 Air bubble fraction (%)	614 DSC determined T_m
615 speed	616 (m/s)	617 elastic modulus (MPa)	618 modulus (kPa)	619	620 ($^{\circ}$ C)
621 1%-no mix	622 1667 ± 83.4	623 2.8×10^3	624 5.0 ± 0.025	625 Negligible	626 77.2
627 1%-slow mix	628 1775 ± 86.4	629 3.2×10^3	630 5.8 ± 0.029	631 8.67 ± 0.44	632 80.6
633 1%-fast mix	634 1925 ± 64.2	635 3.8×10^3	636 6.6 ± 0.033	637 0.59 ± 0.02	638 84.2
639 3%-no mix	640 1790 ± 83.2	641 3.3×10^3	642 99.5 ± 6.67	643 Negligible	644 84.5
645 3%-slow mix	646 1895 ± 92.1	647 3.7×10^3	648 116 ± 3.8	649 4.49 ± 0.23	650 85.5
651 3%-fast mix	652 2025 ± 95.3	653 4.2×10^3	654 132 ± 3.5	655 0.49 ± 0.02	656 86.6

673 numbers of the same size air bubbles while an increase in
 674 mixing speed generally promotes larger numbers of smaller
 675 bubbles (Hanselmann & Windhab, 1999; Scanlon & Zghal,
 676 2001). However, Hanselmann and Windhab (1999) noted
 677 that mixing speed cannot be taken as a reference in bubble
 678 generation. This can be explained by noting that the flow
 679 field around a mixer at similar speeds can vary. Results
 680 presented in Table 1 seem to offer further support to the
 681 above explanation. From Table 1, it appears that the effect
 682 of mixing on bubble size is dependent on the rheology of the
 683 system. There seems to be a greater difference in induced air
 684 bubble fraction between the slow and fast mixing treatments
 685 for the 1% agar gel than the 3% agar gel. That seems to be a
 686 logical result as the larger viscosity of the agar pastes for the
 687 high concentration systems may avoid the formation of a
 688 larger number and large size air bubbles. These results were
 689 corroborated by microscopy. Fig. 4 illustrates photomicrographs
 690 of the 3% fast mixed agar gel (a) and the 3% agar
 691 slow mixed agar gel (b). ANOVA performed at $P=0.05$
 692 indicated that there was no significant differences in the
 693 average bubble sizes obtained for the fast and slow mixed
 694 3% agar gels, which were 0.14 and 0.22 mm, respectively.
 695 Fig. 5 shows photomicrographs of the 1% fast mixed agar
 696 gel (Fig. 5a) and the 1% slow mixed agar gel (Fig. 5b).
 697 As determined by ANOVA at $P=0.05$, the average bubble
 698 sizes were significantly different, 0.12 versus 0.37 mm.
 699 There seem to be a greater effect of mixing speed on bubble
 700 size at lower gel concentrations and again it can be noted
 701 that mixing speed cannot be taken alone as a reference in
 702 bubble generation (Hanselmann & Windhab, 1999).
 703 These results clearly show that mixing speed seemed to



727 Fig. 4. Micrographs showing the air bubbles characteristics in a 1% agar
 728 gel. (a) Fast mixing conditions (b) slow mixing conditions.



729 Fig. 5. Micrographs showing the air bubbles characteristics in a 3% agar
 730 gel. (a) Fast mixing conditions (b) slow mixing conditions.

731 have more of an affect on the bubble size at lower
 732 concentrations.

733 Both the ultrasound derived elastic modulus values
 734 (obtained by using Eq. (2)) and the compressive elastic
 735 modulus values of the agar gels from the quasi-static
 736 compression tests are shown in Table 1. Again it is observed
 737 that both methods yielded larger elastic moduli for the agar
 738 gels receiving the fastest mixing speed. Thus, there is an
 739 agreement with the results obtained from both methods.
 740 However, the absolute values of the elastic moduli obtained
 741 from the different methods differ by nearly four orders of
 742 magnitude. An explanation of this is based of the work of
 743 Kudryashov, Hunt, Arikainen, and Buckin (2001) who
 744 compared the rheological properties of milk gels obtained
 745 by high frequency ultrasonic techniques and low frequency
 746 dynamic rheology. They observed that the values of storage
 747 modulus obtained from both methods values differed by
 748 several orders of magnitude. Kudryashov et al. (2001) noted
 749 that the major physical differences between high and low
 750 frequency rheological measurements may be due to the time
 751 and length scales of the measurements.

752 Our results using both ultrasonic and conventional
 753 rheology (oscillatory dynamic and quasi-static tests) are
 754 indicating that fastest mixing gels are more elastic.
 755 Ultrasonic velocity was slower in the no mix gels, i.e. the
 756 gels with lower or no air bubble fraction. The no mix gels
 757 had less air in the material and it has been shown that air
 758 slows the ultrasonic velocity (McClements, 1991, 1998).
 759 Therefore, it is reasonable to think that they would have
 760 fastest ultrasonic velocities. On a macroscopic level,
 761 porosity weakens the mechanical properties of a material,

785 implying larger elastic moduli values would be measured in
786 the no mix gels (Sayers, 1993). However, the experimental
787 results are showing the opposite effect. These results may be
788 explained by discussing the nature of agar gels on a
789 microscopic or molecular level.

791 3.3. Relationship between mixing conditions and 792 rheological properties—microstructural considerations

793
794 Agar forms a gel by double helix formation and
795 subsequent aggregation of these helices into bundles called
796 suprahelices (Djabourov et al., 1989; Watase et al., 1989). It
797 is speculated that faster mixing promotes the formation of
798 more suprahelices and hence a stronger gel. This idea of gel
799 strength and gel microstructure being affected by gel
800 preparation is supported by work performed by Kusakawa,
801 Ostrovsky, and Garner (1999) that studied the effect of
802 gelation conditions (e.g. cooling rate) on the microstructural
803 properties and the resolving power of agarose based DNA
804 sequencing gels. They noted that agarose fibers in the more
805 slowly cooled gel were more heterogeneous on a micro-
806 scopic basis and showed thicker bundles of fibers (i.e. more
807 suprahelices). DSC results showed that for gels cooled at
808 low cooling rate the melting temperature transition of the
809 gel was 4–5 °C higher than the melting temperature
810 transition of more rapidly cooled gels. The authors stated
811 that the more slowly cooled gels were more thermodynamically
812 stable and exhibited a higher order structure (i.e. with the
813 presence of more suprahelices) (Kusakawa et al.,
814 1999). These facts are significant and are of relevance with
815 regards to explaining the reason for the faster mixed gels
816 exhibiting stronger rheological properties. It is believed that
817 the faster mix gels would exhibit a more thermodynamically
818 stable higher order structure (i.e. contain more aggregated
819 double helices or suprahelices) than slow mixing or not
820 mixed gels and would exhibit higher melting temperatures
821 when analyzed with DSC. In Table 1, values of the melting
822 point temperatures (T_m) obtained from DSC thermograms
823 for the 1 and 3% gels prepared under different mixing
824 conditions are reported. Values of the melting point
825 temperatures are in the range of values reported by Watase
826 et al. (1989) for their DSC experiments regarding agarose
827 gels. The difference between the melting point temperatures
828 of no mix and the fast mix gels for the 1 and 3% gels were
829 7 and 2 °C, respectively. Watase et al. (1989) also noted that
830 the melting point temperatures increased with increasing gel
831 concentration, which is observed in our results. In addition
832 gels of the same concentration when mixed at higher speeds,
833 exhibit both stronger mechanical properties and higher
834 melting point temperatures.

835 For clarity, it should be noted that the following
836 discussion pertains to the microscopic aspects (suprahe-
837 lices) of the gels and how they are affected by the mixing
838 speed on gels and not their macroscopic aspects (air
839 bubbles). Even an unmixed gel is a porous media (i.e. a
840 Biot media) consisting of open pores (spaces between

the grains of the gel) and therefore exhibits porosity. It is
these open pores and degree of porosity that influences the
chromatographic or resolving power of a gel, which is
broadly defined as the limit of the sizes of molecules that
can fit through the pores. This statement is further supported
by the work of Kusakawa et al. (1999) in which
transmission electron micrographs demonstrated a differ-
ence in pore structure between rapidly cooled and slowly
cooled gels. The slowly cooled agarose gels exhibited larger
average pore diameters and therefore a more open structure.
They noted that pore structure would affect the resolving
properties of gels. Both the slowly cooled and rapidly
cooled gels in their experiments were prepared at the same
concentration and therefore the gels had the same total mass
to volume ratio. They hypothesize that the more slowly
cooled gels were taking on a higher order structure (i.e.
more suprahelices). DSC experiments helped them to
corroborate their hypothesis in that the more slowly cooled
gels exhibited higher T_m than the rapidly cooled agarose
gels. Also, work by Brahasandra, Burke, Mastrangelo, and
Burns (2001) showed that the electrophoretic mobility of a
migrating molecule is affected by the size of the migrating
molecule, the thickness of the gel polymers, and the
concentration of the gel polymer.

Nevertheless, the structure of the gel molecules seems to
be the one that may affect the resolving power of these agar
systems. In close relation to this, our results showed that the
structure of the gels is affected by mixing speed. Although,
high-resolution microscopy was not performed in our work
on the effect of mixing on the structure of agar gels, DSC
was performed. Results showed that the fast mixed gels
exhibited higher T_m values which may indicate the faster
mixed agar gels taking on a higher order structure than the
more slowly mixed agar gels. Thus, the simple preparation
step of mixing may affect experimental results with regards
to the application to gel electrophoresis. This statement is
made with respect to the fact that our results are showing
that mixing speed affects the degree of association between
agar molecules, which in turn may affect the resolving
power of these gels. In addition the higher elastic modulus
of gel mixed at faster speeds could be positively related to
gel concentration and this may also affect the resolving
powers of these gels. Noting the effect of mixing may help
to reduce inter-experimental and inter-laboratory variation
and again highlights the importance of gel preparation
techniques.

4. Conclusions

This work has shown the importance of a simple
preparation step of mixing on the mechanical properties
and ultimately the utility/functionality of agar gels. Ultra-
sound velocity measurements were used to deduce rheologi-
cal characteristics of an agar gel. Both ultrasound derived
and conventional rheological measurements of mechanical

properties may be more sensitive to the microstructural aspects of a system versus the macrostructural aspects of a system.

References

- Arnott, S., Fulmer, A., & Scott, W. E. (1974). The agarose double helix and its function in agarose gel structure. *Journal of Molecular Biology*, *90*, 269–284.
- Attenburrow, G. F., Goodbrand, R. M., Taylor, L. J., & Liliford, P. J. (1989). Structure, mechanics and texture of a food sponge. *Journal of Cereal Science*, *9*, 61–70.
- Beer, F. P., & Johnson, E. R. (1992). *Mechanics of materials*. New York: McGraw-Hill.
- Benedito, J., Carcel, J. A., Gonzalez, R., & Sanjuan, N. (2000). Prediction of instrumental and sensory textural characteristics of Mahon cheese from ultrasonic measurements. *Journal of Texture Studies*, *31*, 631–643.
- Brahmasandra, S. N., Burke, D. T., Mastrangelo, C. H., & Burns, M. A. (2001). Mobility, diffusion, and dispersion of single stranded DNA in sequencing gel. *Electrophoresis*, *22*, 1046–1062.
- Campanella, O. H., & Peleg, M. (1987). A note on the relationship between the dynamic viscosity and the relaxation modulus of viscoelastic liquids. *Journal of Rheology*, *31*(6), 511–513.
- Campbell, G. M., Rielly, C. D., Fryer, P. J., & Sadd, P. A. (1998). Aeration of bread dough during mixing: effect of mixing dough at reduced pressure. *Cereal Food World*, *43*(3), 163–167.
- Chambon, F., & Winter, H. H. (1987). Linear viscoelasticity at the gel point of a crosslinking PDMS with imbalanced stoichiometry. *Journal of Rheology*, *31*(8), 683–697.
- Clark, A. H., & Ross-Murphy, S. B. (1985). The concentration dependence of biopolymer gel modulus. *British Polymer Journal*, *17*(2), 164–168.
- Clayton, J. T., & Huang, C. T. (1984). Porosity of extruded foods. In B. M. McKenna (Ed.), *Engineering and food* (Vol. 2), 611–620.
- Djabourov, M., Clark, A. H., Rowlands, D. W., & Ross-Murphy, S. B. (1989). Small angle X-ray scattering characterization of agarose sols and gels. *Macromolecules*, *22*, 180–188.
- Eldridge, J. E., & Ferry, J. D. (1954). Studies of the cross-linking process in gelatin gels III. Dependence of melting point on concentration and molecular weight. *Journal of Physical Chemistry*, *58*, 992–995.
- Gibson, L. J., Ashby, M. F., Schajer, G. S., & Robertson, C. I. (1982). The mechanics of two dimensional cellular solids. *Proceedings of the Royal Society of London. Series A, Mathematical and Physical Sciences*, *A382*(1782), 25–42.
- Hanselmann, W., & Windhab, E. (1999). Flow characteristics and modeling of foam generation in a continuous rotor/stator mixer. *Journal of Food Engineering*, *38*, 393–405.
- Hermans, J. (1965). Investigation of the elastic properties of the particle network in gelled solutions of hydrocolloids. I. Carboxymethyl cellulose. *Journal of Polymer Science. Part A*, *3*, 1859–1868.
- Inoue, K., Song, Y. X., Kamiunten, O., Oku, J., Terao, T., & Fugii, K. (2002). Effect of mixing method on rheological properties of alginate impression materials. *Journal of Oral Rehabilitation*, *29*, 615–619.
- Kudryashov, E. D., Hunt, N. T., Arikainen, E. O., & Buckin, V. A. (2001). Monitoring acidified milk gel formation by ultrasonic shear wave measurements: high frequency viscoelastic moduli of milk and acidified milk. *Journal of Dairy Science*, *84*, 375–388.
- Kusukawa, N., Ostrovsky, M. V., & Garner, M. M. (1999). Effect of gelation conditions on the gel structure and resolving power of agarose-based DNA sequencing gels. *Electrophoresis*, *20*, 1455–1461.
- Labropoulos, K. C., Niesz, D. E., Danforth, S. C., & Kevrekidis, P. G. (2002). Dynamic rheology of agar gels: theory and experiments part I development of a rheological model. *Carbohydrate Polymers*, *50*, 393–406.
- McClements, D. J. (1991). Ultrasonic characterization of emulsions and suspensions. *Advances in Colloidal and Interface Sciences*, *37*, 33–72.
- McClements, D. J. (1998). Particle sizing of food emulsions using ultrasonic spectrometry: Principles, techniques and applications. In M. J. W. Povey, & T. J. Mason (Eds.), *Mason ultrasound in food processing* (pp. 85–104). London: Blackie Academic and Professional.
- Mitchell, J. R. (1976). Rheology of gels. *Journal of Texture Studies*, *7*, 313–339.
- Moritaka, H., Nishinari, K., & Horiuchi, H. (1980). Rheological properties of aqueous agarose–gelatin gels. *Journal of Texture Studies*, *11*, 257–270.
- Morris, V. J., & Chilvers, G. R. (1983). Rheological studies of specific cation forms of kappa carrageenan gels. *Carbohydrate Polymers*, *3*, 129–141.
- Nussinovitch, A., Ak, M. M., Normand, N. D., & Peleg, M. (1990). Characterization of gellan gels by uniaxial compression, stress relaxation and creep. *Journal of Texture Studies*, *21*, 37–49.
- Oakenfull, D. (1984). A method for using measurements of shear modulus to estimate the size and thermodynamic stability of junction zones in non-covalently cross-linked gels. *Journal of Food Science*, *49*(1110), 1103–1104.
- Povey, M. J. W., & McClements, D. J. (1988). Ultrasonics in food engineering part I: Introduction and experimental methods. *Journal of Food Engineering*, *8*, 217–245.
- Sayers, C. M. (1993). Ultrasound in solids with porosity, microcracking, and polycrystalline structures. In J. D. Achenbach (Ed.), *Evaluation of materials and structures by quantitative ultrasonics* (pp. 250–300). Berlin: Springer, 250–300.
- Scanlon, M. G., & Zghal, M. C. (2001). Bread properties and crumb structure. *Food Research International*, *34*, 841–864.
- Watase, M., Nishinari, K., Clark, A. H., & Ross-Murphy, S. B. (1989). Differential scanning calorimetry, rheology, X-ray, and NMR of very concentrated agarose gels. *Macromolecules*, *22*, 1196–1201.
- Winter, H. H., & Chambon, F. (1986). Analysis of linear viscoelasticity of a crosslinking polymer at the gel point. *Journal of Rheology*, *30*(2), 367–382.