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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.

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

Agar gels have a number of practical applications. In laboratory work, agar gels act as bacterial culture supports, separation media in column chromatography and electrophoresis (Djabourov et al., 1989) and therefore their properties must be maintained within very narrow specifications. Agar gels have found use in medicine and pharmacy (Watase, Nishinari, Clark, & Ross-Murphy, 1989). In the food industry, agar gels are commonly used as thickening and gelling agents (Djabourov et al., 1989; Watase et al., 1989). The physicochemical, mechanical, and rheological properties of agar gels are extremely important. It is also important to be aware of factors that may affect the properties of agar gels. A key reason for the function of agar gels is that they have high elastic moduli at low concentrations (Djabourov et al., 1989). Work has been performed by many authors, which cite the direct relationship between gel concentration and the strength of the gel Djabourov et al., 1989; Moritaka, Nishinari, & Horiuchi, 1980; Watase et al., 1989). Agar forms a thermoreversible gel in aqueous solution and the gel remains stable over a wide temperature range; agar gels have a gelling/setting temperature close to 40 °C and a melting temperature near 90 °C (Djabourov et al., 1989; Moritaka et al., 1980). Literature indicates a direct relationship between gel concentration and gel melting temperature (Eldridge & Ferry, 1954; Moritaka et al., 1980).

These statements are not novel and have long been accepted in the literature concerning the properties of agar and other polysaccharide-derived gels. However, more recent work from Inoue et al. (2002) on alginate based materials used in clinical dentistry indicated that there is a noticeable effect of the mixing method on the rheological properties of the unset alginate pastes and the set alginate materials. Their work indicated that when the alginate pastes were mixed with high-speed rotary mixers, the set alginate materials exhibited greater gel strength than the set alginate materials prepared with a slow speed hand-mixing technique. Inoue et al. (2002) attributed this result to the fact that the high-speed rotary mixed set alginate materials may contain less air bubbles than the set alginate materials prepared with a hand-mixing technique. Inoue et al. (2002) proposed that this seems to indicate that air bubbles are removed during fast speed mixing as a result of the difference in density of the paste and the air as the paste is rotated at high speeds. However, it was also noted by Inoue et al. (2002) that there are many ambiguous properties regarding the effect of mixing on alginate pastes (unset alginate materials) and set alginate materials. Thus, a discussion on the concepts of mixing and air bubbles with regard to well-studied systems is warranted.

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

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

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

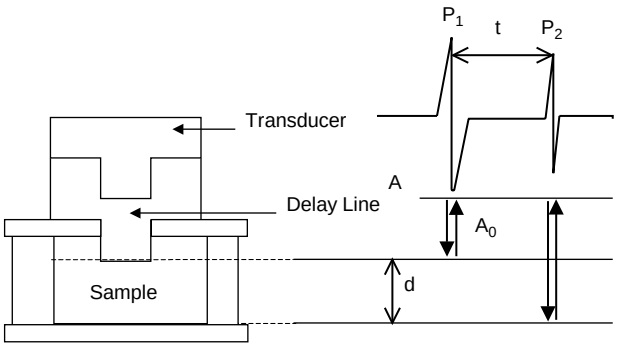

Fig. 1. Schematic of ultrasound set-up.

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)

$$V = \frac{2d}{t} \tag{1}$$

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:

$$V = \sqrt{\frac{E}{\rho}} \tag{2}$$

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 place 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 bubbles 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

$$E = \frac{\sigma}{\varepsilon} \tag{3}$$

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 strereomicroscope (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

$$\rho = \frac{m}{v} \tag{4}$$

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)

$$\phi = 1 - \frac{\rho_b}{\rho_s} \tag{5}$$

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).

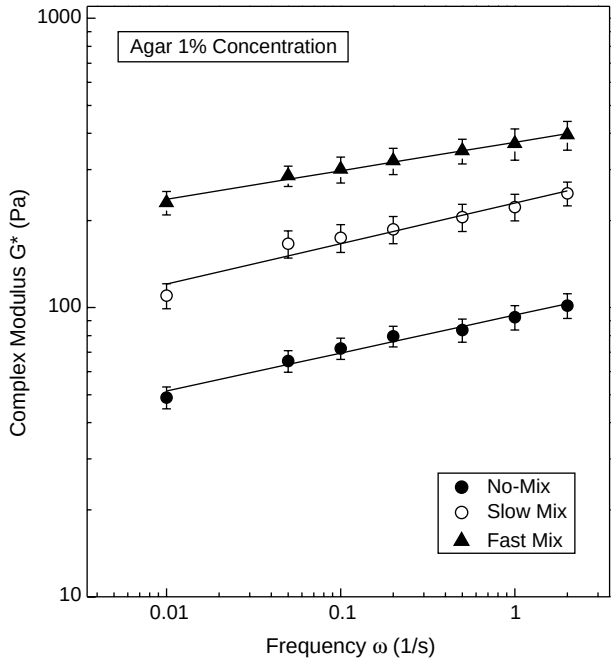

Fig. 2. The effect of mixing speed on the complex modulus for a 1% agar gel.

The power law model can be expressed as:

$$G^* = a\omega^n \tag{6}$$

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

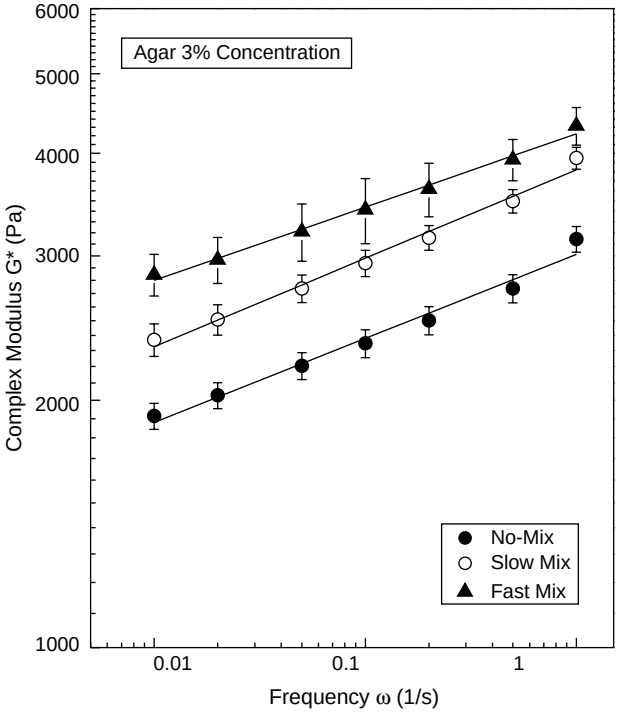

Fig. 3. The effect of mixing speed on the complex modulus G^* for a 3% agar gel.

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

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

3.2. Relationship between mixing conditions and material properties as determined by ultrasound and compression tests—macrostructural considerations

Before discussing the results of uniaxial compression elastic modulus values and ultrasound derived elastic modulus values presented in Table 1, the conclusions obtained in the previous section in regard to the viscoelastic nature of the samples tested will be considered for the analysis of these results. Conventional small strain oscillatory rheometry has shown that the samples could be considered as approximately elastic materials. It is noted that there may be questions to the validity of this assumption as gels can be considered to be viscoelastic materials, in that they possess both elastic and viscous components, but our results are indicating that the elastic component of the studied gels exceed by far their viscous components. Large values of the storage modulus values G' as compared to the measured loss modulus values G'' (data not shown) would be indicating the elastic nature of these gels. Furthermore, based on the work of Benedito, Carcel, Gonzalez, and Sanjuan (2000) it may be valid to consider a gel to be an elastic material and therefore measurement of the elastic modulus through uniaxial compression tests and longitudinal velocity measurements may be suitable. Generally, the bulk modulus of a gel is 2×10^9 Pa, while the shear modulus of a gel is 1000 Pa. The bulk modulus is nearly six orders of magnitude larger than the shear modulus. Based on these facts Benedito et al. (2000) noted that gels could be considered elastic materials and consequently any changes in ultrasound velocity would be mainly due to changes in the bulk or the elastic modulus of the material.

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

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

As already mentioned, the increase in air volume achieved by mixing is biased towards creating larger numbers of the same size air bubbles while an increase in

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

Gel conc. and mix speed	Velocity (m/s)	Ultrasound derived elastic modulus (MPa)	Compressive elastic modulus (kPa)	Air bubble fraction (%)	DSC determined T_m (°C)
1%-no mix	1667±83.4	2.8×10^3	5.0±0.025	Negligible	77.2
1%-slow mix	1775±86.4	3.2×10^3	5.8±0.029	8.67±0.44	80.6
1%-fast mix	1925±64.2	3.8×10^3	6.6±0.033	0.59±0.02	84.2
3%-no mix	1790±83.2	3.3×10^3	99.5±6.67	Negligible	84.5
3%-slow mix	1895±92.1	3.7×10^3	116±3.8	4.49±0.23	85.5
3%-fast mix	2025±95.3	4.2×10^3	132±3.5	0.49±0.02	86.6

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

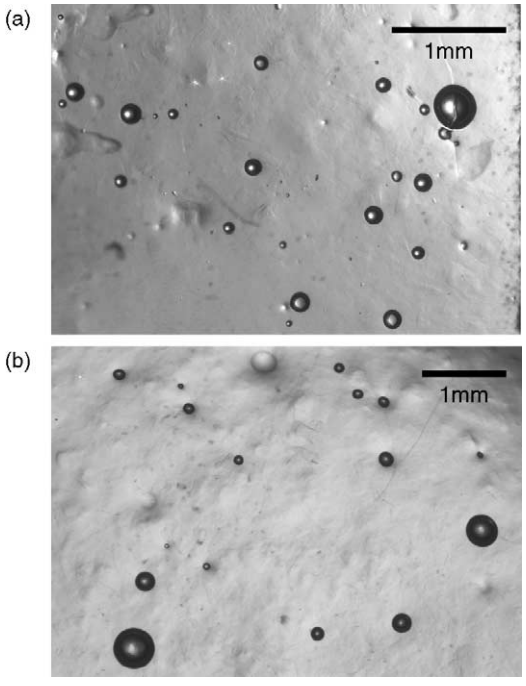

Fig. 4. Micrographs showing the air bubbles characteristics in a 3% agar gel. (a) Fast mixing conditions (b) slow mixing conditions.

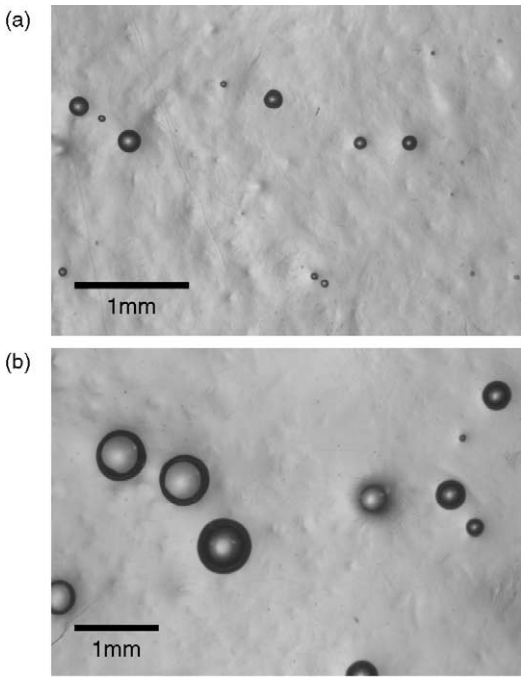

Fig. 5. Micrographs showing the air bubbles characteristics in a 1% agar gel. (a) Fast mixing conditions (b) slow mixing conditions.

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

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

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

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

Agar forms a gel by double helix formation and subsequent aggregation of these helices into bundles called suprahelices (Djabourov et al., 1989; Watase et al., 1989). It is speculated that faster mixing promotes the formation of more suprahelices and hence a stronger gel. This idea of gel strength and gel microstructure being affected by gel preparation is supported by work performed by Kusukawa, Ostrovsky, and Garner (1999) that studied the effect of gelation conditions (e.g. cooling rate) on the microstructural properties and the resolving power of agarose based DNA sequencing gels. They noted that agarose fibers in the more slowly cooled gel were more heterogeneous on a microscopic basis and showed thicker bundles of fibers (i.e. more suprahelices). DSC results showed that for gels cooled at low cooling rate the melting temperature transition of the gel was 4–5 °C higher than the melting temperature transition of more rapidly cooled gels. The authors stated that the more slowly cooled gels were more thermodynamically stable and exhibited a higher order structure (i.e. with the presence of more suprahelices) (Kusukawa et al., 1999). These facts are significant and are of relevance with regards to explaining the reason for the faster mixed gels exhibiting stronger rheological properties. It is believed that the faster mix gels would exhibit a more thermodynamically stable higher order structure (i.e. contain more aggregated double helices or suprahelices) than slow mixing or not mixed gels and would exhibit higher melting temperatures when analyzed with DSC. In Table 1, values of the melting point temperatures (T_m) obtained from DSC thermograms for the 1 and 3% gels prepared under different mixing conditions are reported. Values of the melting point temperatures are in the range of values reported by Watase et al. (1989) for their DSC experiments regarding agarose gels. The difference between the melting point temperatures of no mix and the fast mix gels for the 1 and 3% gels were 7 and 2 °C, respectively. Watase et al. (1989) also noted that the melting point temperatures increased with increasing gel concentration, which is observed in our results. In addition gels of the same concentration when mixed at higher speeds, exhibit both stronger mechanical properties and higher melting point temperatures.

For clarity, it should be noted that the following discussion pertains to the microscopic aspects (suprahelices) of the gels and how they are affected by the mixing speed on gels and not their macroscopic aspects (air bubbles). Even an unmixed gel is a porous media (i.e. a 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 Kusukawa et al. (1999) in which transmission electron micrographs demonstrated a difference 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 Brahmasandra, 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. Ultrasound velocity measurements were used to deduce rheological 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.

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