Viscoelastic properties of high pressure and heat induced tofu gels

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Abstract

Tofu gels were rheologically examined to determine their storage or elastic (G') and loss or viscous (G'') moduli as a function of frequency within their linear viscoelastic limits. The tofu gels were made using either glucono-δ-lactone (GDL) or calcium sulphate ($CaSO_4 \cdot 2H_2O$), followed by either heat treatment (heated soymilk at $\geqslant 97\,°C$ prior to coagulation and subsequently held at $70\,°C$ for 60 min, HT) or high pressure treatment (400 MPa at $20\,°C$ for 10 min, HP). The overall moduli values of the GDL gels and $CaSO_4 \cdot 2H_2O$ gels of both physical treatments were similar, each gave frequency profiles expected for weak viscoelastic materials. However, although both temperature and high pressure treatments could be used to produce tofu gels, the final products were not the same. Pressure formed gels, despite having a higher overall "consistency" (increasing values of their moduli), had a proportionately higher contribution from the loss modulus (increased $\tan \delta$). Differences could also be observed using confocal scanning laser microscopy. While such treatment may give rise to differing systems/structures, with new or modified organoleptic properties, the more "open" structures obtained by pressure treatment may well cause processing difficulties if subsequent reworking or moulding is required.
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1. Introduction

Tofu is usually considered to be a salt- or acid-coagulated soy protein water based gel, with soy lipids and other constituents trapped in its network. The majority of proteins in soybean are glycinin (11S globulin) and β-conglycinin (7S globulin), which account for 65–80% by weight of the total seed proteins present (Liu, 1999). The glycinin and β-conglycinin can be induced to form gels by heat and/or coagulant addition as in commercial tofu production (Fukushima, 1991; Liu, 1999).

A two-step mechanism for the gelation of tofu has been proposed by Kohyama, Sano, and Doi (1995). The first step is usually the heat-induced denaturation of the soy proteins which exposes the hydrophobic regions of the protein molecules. The denatured soy protein is negatively charged (Kohyama & Nishinari, 1993) and any subsequent change in ionic conditions (induced by either GDL or calcium ion addition) neutralises this net charge on the protein. As a consequence of this the hydrophobic interactions of the neutralised protein becomes more dominant and induces the random aggregation of proteins. This in turn leads to gel formation (deMan, deMan, & Gupta, 1986). The lipids are believed to be incorporated into the tofu gel by interactions with the protein particles during the soymilk gelation (Shun-Tang, Ono, & Mikami, 1999). The unique textures of soy protein foods such as tofu are believed to be a result of both the intermolecular interchange between the exposed –SH and S–S groups and the intermolecular hydrophobic interactions among the exposed hydrophobic amino acid residues of the denatured protein (Fukushima, 1991).

Calcium sulphate and glucono-δ-lactone (GDL) are both coagulants widely used for tofu making. While

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calcium sulphate has the ability to incorporate more water into the final tofu, it is difficult to mix well in the soymilk, due to its limited solubility, often resulting in tofu of poor consistency and with an unacceptable texture (Liu, 1999). GDL, an acid coagulant, in contrast has the advantage of forming homogenous "strong" gels (Kohyama & Nishinari, 1993; Liu, 1999). Coagulation by GDL proceeds as a result of heat-activated hydrolysis of lactone into gluconic acid (Liu, 1999). Gelation and subsequent changes in the viscoelasticity of the soybean proteins after addition of either GDL or calcium sulfate have been shown to follow first-order reaction kinetics (Kohyama et al., 1995).

High pressure is a physical means by which to form soy protein gels (Dumoulin, Ozawa, & Hayashi, 1998; Molina & Ledward, 2003; Okamoto, Kawamura, & Hayashi, 1990). The final nature of high pressure induced gels may well be very different to those induced by heat, since the order and extent of any bond breaking and reformation is also likely to be different. High pressure will bring about changes in hydrophobic interactions, hydrogen bonding and electrostatic interactions while covalent bonds are unlikely to be directly affected by pressure (Ledward, 1995). Therefore, high pressure can replace the traditional heat treatment and improve the strength of tofu gel by forming a more cross-linked network (Zhang, Li, Tatsumi, & Isobe, 2005). Compared with the conventional heat process, the high pressure process is considered to be energy efficient, safe and has no environmental pollution.

Rheology is the science of the deformation and flow of matter (Steffe, 1996). Dynamic oscillatory testing techniques are usually non-destructive and have a unique advantage for characterising food systems in their "undisturbed" states (Bell, 1989). Apichartsrangkoon (2003) reported that the rheological properties of soy protein gels changed little with temperature and/or pressure treatment.

There have been many studies reported on tofu gels. Almost all of them have dealt with heat treatment processes. In this investigation, heat and high pressure treatments were used to prepare both GDL and calcium sulphate type tofu gels from soymilk and their rheological and microstructural characteristics subsequently analysed.

2. Materials and methods

Soybeans were purchased from Morning Foods Ltd., UK; glucono-δ-lactone (GDL, C_6H_{16}) and calcium sulphate ($CaSO_4 \cdot 2H_2O$) were purchased from Sigma and were of analytical grade.

2.1. Preparation of soymilk

Washed soybean was soaked overnight at room temperature (20–25 °C) in 10 times its weight of deionised water. After draining off excess water, five times by weight of deionised water was added to the equivalent dry weight of the soybean material. The mixtures were ground and filtered through a filter cloth to remove any okara, then centrifuged at 1200g and 10 °C for 5 min The soymilk ($4.4 \pm 0.1\%$ protein; $8.7 \pm 0.3\%$ total solid, pH 6.5 ± 0.1) was then collected as the supernatant and kept at 4 °C for further processing. This soymilk was used to produce tofu within one week of production.

2.2. Preparation of tofu gels

2.2.1. Heat induced tofu gels
Soymilk was heated in a boiling water bath to $\geqslant 97$ °C for 7 min, with regular stirring. It was then cooled in an ice bath to room temperature. Subsequently 3 ml of GDL solution or calcium sulfate suspension was added to 100 ml of "cooked" soymilk, at room temperature, to make a final concentration of 0.4% w/v GDL or 0.4% w/v $CaSO_4 \cdot 2H_2O$. This mixture was then uniformly mixed and heated in water bath at 70 °C for 60 min The samples were subsequently cooled in an ice bath to room temperature and held at room temperature for 60 min prior to storage at 4 °C.

2.2.2. High pressure induced tofu gels
A solution of 3 ml GDL or calcium sulphate suspension was added to 100 ml of raw soymilk at room temperature, to make a final concentration of 0.4% w/v coagulant (as previously). The mixture was then uniformly mixed and subsequently sealed in a "Cryovac" plastic bag and subjected to a pressure of 400 MPa at room temperature for 10 min using a "Food lab" high pressure rig (Stansted Fluid Power, Essex, UK). The pressurisation rate was about 250 MPa/min, using a mixture of castor oil and ethanol (20:80) as the pressure transmission media. Initial temperature of the pressure vessel was 20 °C, but due to the adiabatic effect and the pressure used, the temperature rose to maximum of 36 °C and subsequently, within 2 min, fell back to room temperature (20 °C). After treatment, samples were aged at room temperature for 60 min prior to storage at 4 °C.

2.3. Rheological measurements

A controlled stress rheometer (Rheo-Tech International Ltd., Royston, England) was used to measure the dynamic viscoelastic properties of the samples. (Measuring geometry used, 20 mm diameter parallel plate, gap width 2 mm). Samples were loaded into the rheometer and allowed to equilibrate to the measuring temperature (25 °C). Excess sample was carefully removed with a razor blade and a thin layer of low viscosity (less than 1 cp) silicone lubricant was applied to the exposed free edges to prevent evaporation of water. Subsequent examination of the samples showed no ingress of lubricant.

The linear viscoelastic regions of the various samples were determined by means of oscillation stress sweeps (0.5–10 Pa at a frequency of 1 Hz). Based on these results, a stress amplitude of 1 Pa was chosen for measuring the frequency dependent moduli of both the HT and the HP

treated samples. This was well within the linear viscoelastic region of behaviour for all of samples used in this study (data not shown). Storage (G') and loss (G'') moduli were obtained by oscillation sweep measurement over a frequency range of 0.01–10 Hz. Each profile shown was the average of six sample preparations.

2.4. Confocal scanning laser microscopy (CSLM)

Sections of samples were cut with a razor blade, placed on a microscope slide and a drop of a fluorescent dye mixture of both Fast green FCF and Nile Red solution, dissolved in polyethyleneglycol 200 was added (Auty, Twomey, Guinee, & Mulvihill, 2001). CSLM work was performed using a Leica DM IRE 2 Confocal Scanning Microscope configured with an inverted microscope using a Helium/Neon laser filtering emission (625–754 nm). Images of representative areas of each sample were taken using a suitable magnification objective (×63).

2.5. Water holding capacity

Water holding capacity (WHC) determinations were based on the method proposed by Molina, Defaye, and Ledward (2002) with slight modification. Cylindrical portions (about 1 cm length and 9 mm diameter) of the tofu gels were placed into a centrifugal filter system (Amicon Ultrafree®-Cl, pore diameter 0.45 μm). A centrifugal force of 4000g was applied for 10 min at 15 °C (Sorvall® RC 5 B plus, DuPont) and the weight of released water was subsequently measured. The results are the means from six sample replications. WHC was calculated as the percentage of water released per gram weight of the original sample used.

2.6. Statistical analysis

All data reported represent the mean ± standard deviation ($n = 6$). An analysis variance (ANOVA) of the data was performed and a least significant difference (LSD) test with a confidence interval of 95% was used to compare the means.

3. Results and discussion

The visual appearance of 0.4% w/v GDL and 0.4% w/v $CaSO_4 \cdot 2H_2O$ gels, induced by heat, were essentially smooth and homogenous and had good water retaining properties (Fig. 1). The heated soymilk, without added coagulant remained liquid even after processing under the same conditions. This suggested that the untreated soymilk had too low a protein concentration to form a gel without a coagulant. It has been reported that a high protein concentration (greater than 7% w/w) is needed to form a self-supporting soy protein gel by heat processing alone (Hermansson, 1978).

Under conditions of high pressure treatment, soymilk without coagulant remained liquid after processing at both
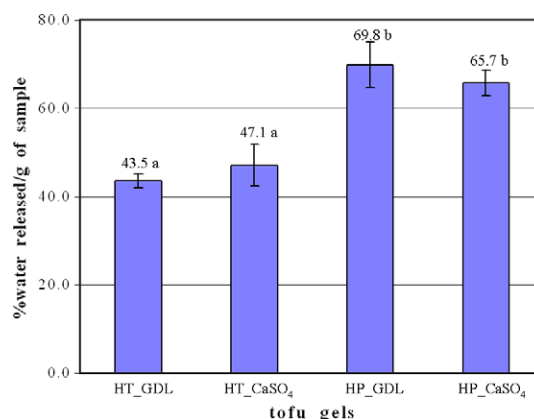

Fig. 1. Water holding capacity (WHC) of the heat (HT) and high pressure (HP) induced GDL and $CaSO_4 \cdot 2H_2O$ tofu gels (measurement of six replications). Mean followed by the same letters are not significantly different ($P < 0.05$).

400 and 600 MPa with temperatures of both 20 and 40 °C for 40 min Soymilk with added 0.4% w/v GDL or 0.4% w/v calcium sulphate when processed at 400 MPa and 20 °C for 10 min both gave gel systems. These when compared with the equivalent HT materials (same amount of coagulant), were found to have a very "loose" texture, which on compression exuded liquid and underwent progressive fluid loss with time. Tofu gels with the same concentration of coagulant (0.4% w/v GDL or 0.4% w/v calcium sulphate), processed at higher pressures, longer times or higher temperatures, produced gels with "tougher" and more "crumbly" textures.

Both the GDL and $CaSO_4 \cdot 2H_2O$ tofu gels from both heat and high pressure treatment were examined as a function of stress, at a fixed frequency (1 Hz) and were shown to behave in a linear viscoelastic manner well above the stresses used for subsequent rheological measurements. In such a linear region of viscoelastic behaviour the rheological properties are essentially strain or stress independent (Steffe, 1996).

Figs. 2 and 3 show that the storage moduli of both the heat and high pressure induced GDL and $CaSO_4 \cdot 2H_2O$

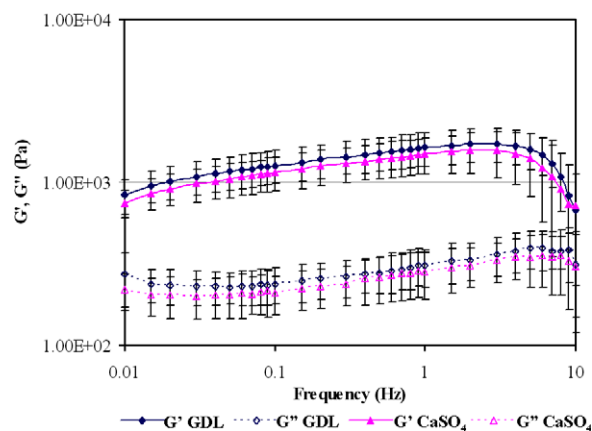Fig. 2. The storage (G') and loss (G'') moduli of heat induced tofu gels with 0.4% (w/v) GDL and 0.4% (w/v) $CaSO_4 \cdot 2H_2O$ as a function of frequency (0.01–10 Hz) at a stress amplitude of 1 Pa ($n = 6$).
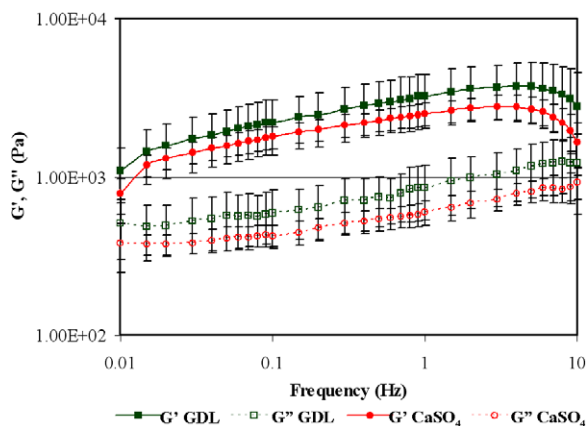
Fig. 3. The storage (G') and loss (G'') moduli of high pressure (400 MPa, 10 min) induced tofu gels with 0.4% (w/v) GDL and 0.4% (w/v) $CaSO_4 \cdot 2H_2O$ as a function of frequency (0.01–10 Hz) at a stress amplitude of 1 Pa ($n = 6$).

Table 1
Viscoelastic properties at frequency of 0.1 and 1.0 Hz for heat treated (HT) and high pressure induced (400 MPa, 10 min), (HP) tofu gels with both GDL and $CaSO_4 \cdot 2H_2O$ coagulants

	HT		HP	
	GDL	$CaSO_4 \cdot 2H_2O$	GDL	$CaSO_4 \cdot 2H_2O$
0.1 Hz				
G' (kPa)	1.27 ± 0.30 a,b	1.16 ± 0.27 a	2.22 ± 0.83 c	1.77 ± 0.28 b,c
G'' (kPa)	0.24 ± 0.06 a	0.21 ± 0.06 a	0.60 ± 0.23 b	0.43 ± 0.07 b
tan δ	0.19 ± 0.01 a	0.18 ± 0.01 a	0.27 ± 0.02 c	0.24 ± 0.02 b
1.0 Hz				
G' (kPa)	1.64 ± 0.39 a	1.49 ± 0.38 a	3.23 ± 1.19 b	2.52 ± 0.41 b
G'' (kPa)	0.31 ± 0.08 a	0.28 ± 0.09 a	0.86 ± 0.33 b	0.60 ± 0.11 b
tan δ	0.19 ± 0.01 a	0.19 ± 0.01 a	0.27 ± 0.01 c	0.24 ± 0.01 b

The results were the means ± standard deviations ($n = 6$). Same letter indicates no significant difference between the data ($P < 0.05$) within each row.

tofu gels increase slightly with increasing frequency. All gels gave the frequency profiles expected for weak viscoelastic systems for which the elastic or storage modulus (G') is greater than the viscous or loss modulus (G''), over most of the measured frequency range. Such profiles are consistent with the behaviour of a material with weak non-specific interactions giving rise to amorphous gel structures (Ferry, 1980).

The frequency dependence of the storage and loss moduli for all the differing types of gels produced was essentially the same. These similar rheological profiles suggested that although the absolute "strength" of the gels may vary, they were essentially the same type of rheological system, formed by the non-specific cross linking of the denatured protein material. The differences between the GDL induced and the calcium sulphate induced gelation systems were mainly those observed in the gelation rate, that is gelation by calcium sulphate being faster (Kohyama et al., 1995).

The HP induced gels produced with both GDL and calcium sulphate give consistently higher values of both the storage (G') and the loss (G'') moduli than those produced by HT treatment (Table 1). This suggested that although similar types of network were formed (weak viscoelastic gels) by the different treatments, the HP induced gels were "stronger" or had a greater elasticity than those of the equivalent HT gels.

It was observed that the HP induced gels showed a slight increase in their frequency dependence when compared to the equivalent HT materials. This was accompanied by an increase in the tan δ for the HP induced gels (Table 1). Such behaviour whilst indicative of increased "consistency" suggests that the pressure induced material may represent a more overall "liquid" system [increased contribution from the loss modulus (Ferry, 1980)]. All of the gels showed some structural break down at higher frequencies, especially in the HT induced gels (data not shown due to high s.d. values). Again the onset of such behaviour is

indicative of the underlying structures present with the more "rigid gel like" heat induced material showing damage at slightly "earlier" frequency values. This frequency induced damage is indicative of increased interaction due to changes in the "time scale" of the interactions present in a more structured systems (Ferry, 1980) and is unlikely to be related to any strain damage, as the controlled stress instrument actually generates lower values at the higher frequencies.

Figs. 4 and 5 show confocal microscopic plates from the HT and the HP processed gels, respectively. Both were stained for both fat and protein materials. The green and red[1] areas in the plates represent the proteins and the fats, respectively, while black areas represent the "voids" in the network. Fig. 4 shows the HT induced GDL and $CaSO_4 \cdot 2H_2O$ gels with an essentially homogenous spread of both protein and the associated fat material throughout the structure. While there was some indication of variations in the overall material density, there was no evidence of any major "voids". The structure of the HP induced gels differ from those of the HT induced gels in that while the fats again seems to be associated with the protein material, the overall structure seems to be more "aggregated" producing a loose "network" of material, containing a considerable number of, presumably, liquid filled spaces (Fig. 5). Such "gels" showed considerable syneresis when processed (cut, milled and so on). These results were supported by subsequent studies on the water holding capacity of the gels which showed that the amounts of water released from HP induced gels were higher than those from HT gels for both coagulants (Fig. 1).

The results obtained from the HP treated samples suggest that a "coarse" open network is formed, having poor reworking and water holding properties. It has been proposed that this type of structure occurs when the aggregation of coagulum occurs quickly, compared to the

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[1] For interpretation of color in Figs. 4 and 5, the reader is referred to the web version of this article.
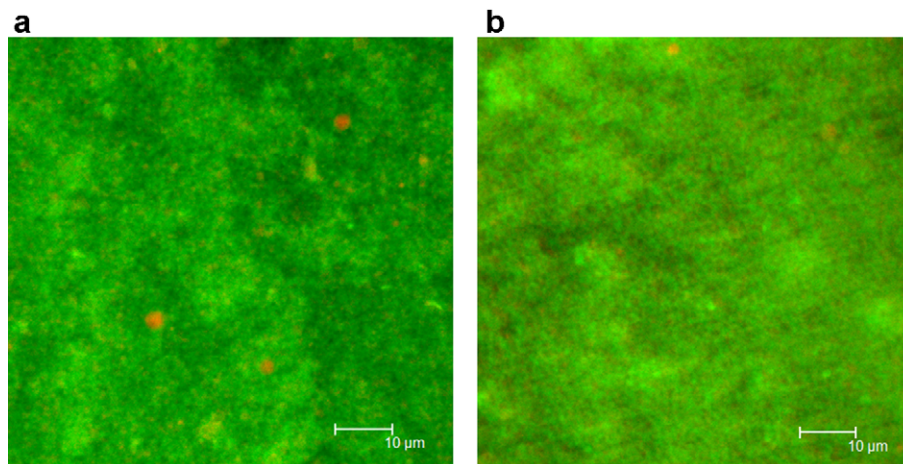
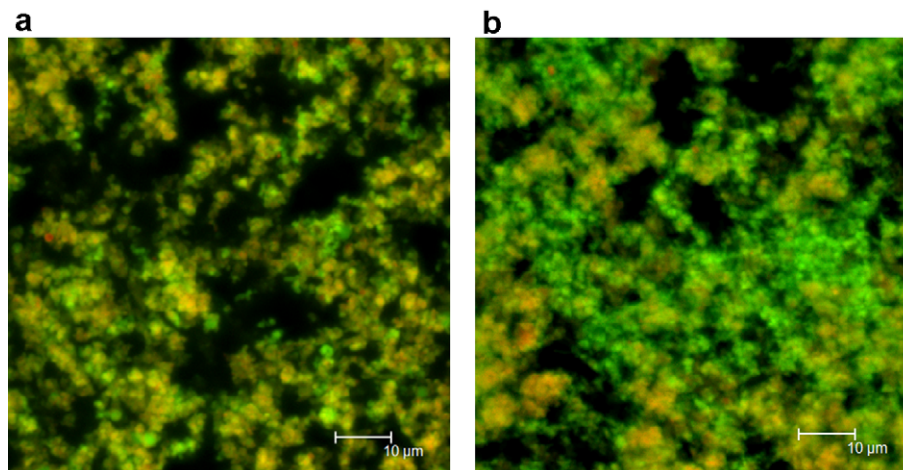Fig. 4. Confocal microscopy image of heat induced tofu gels with 0.4% (w/v) GDL (a) and 0.4% (w/v) CaSO$_4$·2H$_2$O (b).

Fig. 5. Confocal microscopy image of high pressure (400 MPa, 10 min) induced tofu gels with 0.4% (w/v) GDL (a) and 0.4% (w/v) CaSO$_4$·2H$_2$O (b).

denaturation of the protein, so that a random network, that is unable to hold water, is formed and syneresis occurs (Kinsella, Rector, & Philips, 1994). If the aggregation process is slower than the protein denaturation, an ordered structure will be promoted, allowing the denatured molecules to orient themselves in a systematic fashion, prior to aggregation (Hermansson, 1978), as appears to occur on heat treatment (Fig. 4).

4. Conclusions

The present results suggest that although high pressure can be used to produce GDL and CaSO$_4$·2H$_2$O tofu "gels", these products differ from those traditionally produced by heat treatment. The gels produced by pressure, although having a higher overall "consistency" (increased moduli values) seem essentially to be a more "liquid" viscoelastic system (increase in tan δ), than those obtained by heat treatment. The differing structures produced can subsequently be observed using microscopic techniques. The more "open" structure produced by pressure contains con-

siderable "void" areas (Fig. 5) which are absent in the more homogeneous heat treated material (Fig. 4). Such differences may well have considerable implications in terms of product development. While giving rise to differing systems/structures with possibly new or modified organoleptic properties, the more open structure (and associated syneresis), could cause considerable problems if further reworking of the material is required.

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