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The effect of shear on the rheology and structure of heat induced whey protein gels

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Abstract

The influence of mechanical shearing on the small deformation properties and microstructure of heat induced whey protein gel has been studied. The viscoelastic properties of these gels at different concentrations of 10 and 20% (w/w) exposed to different shear rates of 0, 50, 100, 200 and 500s⁻¹ during gelation were measured using dynamic oscillatory rheometry. The structure of both the shear treated and unsheared gels were then investigated using light microscopy. The results showed that the storage modulus of the gels at both concentrations

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was increased by increasing the shear rate exposure during gelation while the shear treated gels were more elastic and showed frequency-independent behaviour. As the total protein concentration of the gel increased, the viscoelastic properties of the gels also increased significantly and the gels showed greater elasticity. The gels obtained from the higher shear rate exposure were stronger with higher elastic moduli at both protein concentrations. Images of the gels obtained using light microscopy showed that shearing resulted in phase separation and some aggregation in the structure of the gels at both concentrations. However, the shearing rates applied in this study were not enough to cause aggregation breakdown in the gel network.

Keywords: Microstructure, Rheology, Shearing, Storage modulus, Whey protein gel

1. Introduction

Globular proteins have range of food applications due to both their nutritional and remarkable functional properties. Gelation is one of the most important functional properties of proteins that may affect the sensory and structure of food products (Totosa, *et al.*, 2002). The mechanism and properties of heat induced protein gels have been fully studied (Doi, 1993, Ikeda and Nishinari, 2001, Gosal and Ross-Murphy, 2000). However, other physical, chemical and enzymatic treatments can induce gelation (Totosa, *et al.*, 2002). Thermal gelation of globular proteins involves two steps: thermal denaturation and unfolding of the proteins, followed by intermolecular aggregation and rearrangement. Different factors such as pH, ionic strength and mechanical stresses can affect the properties and structure of the heat induced gels (Totosa, *et al.*, 2002).

A number of researchers have studied the effects of shear exposure during gelation on the mechanical properties of the gels formed (Moakes *et al.*, 2015, Walkenstrom and Hermansson, 1998, Walkenstrom *et al.*, 1998, Ker and Chen, 1998). The response of proteins to applied shear stresses is an important area of study because many proteins undergo shear stress as an inevitable factor in bioprocessing and physiology. Furthermore, the ability to modify the processing conditions, and hence shear exposure, during gelation, offers a method of modifying the material properties of the gels formed. Many proteins are submitted to mechanical shearing through different food processes such as filtration, mixing, pumping and extrusion (Ker and Chen, 1998). Shearing has been shown to influence the structural conformation of proteins and change the functional and gelation properties of proteins (Ker and Chen, 1998, Dunstan, *et al.*, 2009a, Bekard, *et al.*, 2012, Ow and Dunstan, 2013). Mechanical shearing may modify chemical and enzymatic treatments during processing and

may also improve functional properties of proteins in specific conditions (Ker and Chen, 1998). The effects of shear on conformation and properties of different globular protein have been extensively investigated. At low pH, the protein is highly charged and the application of shear stress promotes the formation of fibrillar structure in globular proteins such as whey protein isolate (Akkermans, *et al.*, 2008), Bovine serum albumin (Bekard, *et al.*, 2012), lysozyme (Ow and Dunstan, 2013), β -lactoglobulin (Dunstan, *et al.*, 2009b, Hill, *et al.*, 2006, Akkermans, *et al.*, 2006), amyloid- β (Hamilton-Brown, *et al.*, 2008, Dunstan, *et al.*, 2009a), and soy proteins (Ker and Chen, 1998, Akkermans, *et al.*, 2007).

Walkenstrom *et al.* (1998) showed that heavily sheared whey protein concentrate formed weak gels with a lower storage modulus than that of the unsheared gels, while the microstructure of shear treated and unsheared gels did not show significant structural differences as observed optically. Their study also showed that the mixed gels of gelatin and whey protein were less sensitive to shearing than the pure gel of whey protein. Ker and Chen (1998) indicated that soy protein gels have a shear thickening property and that high shearing promotes gel formation. However, Moakes *et al.* (2015) showed that whey protein fluid gels had a shear thinning behaviour as a result of particle break up.

The objective of this study was to describe the effect of different shear rates on the rheological properties and microstructure of the heat induced whey protein gel. This understanding will enable whey protein gels to be modified in their mechanical properties and the effect of processing where the whey protein encounters mechanical shearing as a part of the processing and extraction to be understood.

2. Materials and Methods

2.1. Materials

Whey protein isolate powder (NatraPro, MG Nutritionals, Melbourne, Australia) was dissolved in double distilled water to concentrations of 10 and 20% w/w and stirred for 1 h at room temperature. The pH was not adjusted and the solution had a natural pH value of 6.4. Prior to the rheological experiments, whey protein solutions were degassed under vacuum until all visible air bubbles disappeared.

2.2. Dynamic Rheological Measurements

Dynamic rheological measurements were performed on a stress controlled AR-G2 rheometer, TA instruments (UK), using bob and cup configuration with a cup diameter of 30 mm and a bob diameter of 28.06 mm. Degassed whey protein solutions were poured directly into the rheometer's cup, which was preset at 90°C and exposed to different shear rates of 0, 50, 100,

200 and 500 s^{-1} for 2 h. In order to prevent evaporation at high temperature, the sample was immediately covered with paraffin oil. Then the temperature was decreased from 90 to 20°C, at a cooling rate of 2.5°C/min. During the cooling step, the viscoelastic properties of the sample were measured at a constant frequency of 0.1Hz. After this measurement, the sample was held at 20°C for 1 h (Akkermans, *et al.*, 2008). Subsequently, a frequency sweep measurement was performed in the range of 0.1-10 Hz. The strain was 1×10^{-3} , which is in the linear viscoelastic region of the sample and determined by the strain sweep test.

2.3. Microscopy

An Olympus IX71 light microscope (Japan) with 10× Objective and Qimaging camera (Micropublisher), Brightfield imaging was used to examine the structure of the gels formed in the rheometer. Samples were sheared and then the intact gels were removed from the rheometer and transferred directly onto the microscope slide in order to preserve the structure of the gels.

2.4. Statistical analysis

One-way analysis of variance (ANOVA) using SPSS software (SPSS version 22, SPSS Inc., Chicago, USA) was performed in order to test the effect of shearing rates on the viscoelastic properties of heat induced whey protein gels. Duncan's multiple range test was used to determine the differences between means at a significant level of 0.05 ($P < 0.05$).

3. Results and Discussion

The rheological properties of shear treated heat induced whey protein gels were studied at two different concentrations of 10 and 20% (w/w). Whey protein solutions were exposed to different shear rates at a constant temperature of 90°C for 2 hours prior to cessation of the shear and allowing the temperature of the solution to reduce to 20°C while measuring the modulus of the gelling solutions.

The values of G' for the 10% whey protein gel, obtained upon heating and shear treatment, were measured during cooling from 90 to 20°C. Figure 1a shows that the storage modulus of the gels at different shear rates increased significantly over cooling. The formation and development of hydrogen bonding over cooling may result in an increase in the storage modulus of the gels (Chen and Dickinson, 1998). The results show that the structures of the gels become more rigid showing higher values of G' with increasing shear rate. The higher values of G' for the shear treated gels over the cooling period could be explained as a result of shear-induced secondary aggregation phenomena and the structural rearrangement (Walkenstrom and Hermansson, 1998). The same behaviour was observed for 20% whey

protein gels (Figure 1b), as the total protein concentration of the gel increased, the values of G' and G'' of the gels also increased considerably and the gels showed more elastic character (Saglam, *et al.*, 2014). At both concentrations, increasing preshear rates results in the significant increase in the value of G' of the gels obtained during cooling step ($p < 0.05$). Gel strength increased during holding at high temperature mostly due to the formation of disulphide bonds and hydrophobic interactions, while the increase in the value of G' during cooling step was mainly attributed to the formation of hydrogen bonds (Ikeda and Foegeding, 1999).

The frequency dependence of G' and G'' of whey protein gels at concentrations of 10 and 20% are shown in Figure 2a and 2b, respectively. The values of G' and G'' showed similar trends for all the samples at different shear rates with considerably greater values of G' than G'' , showing that essential elastic gels were obtained. The results represent that highly structured gels with the higher elastic modulus than the viscous component were obtained at two concentrations. The values of G' and G'' increased considerably with increasing shear rate and whey protein gel showed viscoelastic properties. It was reported that increasing shear rate during the step of sol-gel transition causes a slowdown in the rate of aggregation and lower degree of particle-particle interactions, which lead to the lower storage modulus for the gels at higher shear rate (Moakes *et al.*, 2015). However, figures 2a and 2b show that the storage modulus increased with exposure to higher shear rates during gelation. Since gelation, in our work, occurred at isothermal high temperature and the protein suspension was exposed to the shearing at high temperature, shear increased the strength of the particle-particle interactions.

All the sheared and unsheared gels showed a solid like mechanical behaviour ($G' > G''$) throughout the experimental frequency range, which is the characteristics of strong and elastic gels (Rocha, *et al.*, 2009, Ross-Murphy, 1995). The values of the moduli for the unsheared gels were less than those of the shear treated gels. The opposite results were reported by Walkenstrom, *et al.*, (1998), where it was found that the G' for the sheared and unsheared whey protein gels were frequency dependent and the values of G' for the unsheared samples were higher than those of the shear treated gels. However, in their research a steady shear treatment was performed on whey protein suspension while it was heated to 82°C, resulted in the aggregation break up under shearing.

Whey protein gels at different shear rates had similar behaviour over a wide range of frequencies. The storage modulus of the gels at both concentrations showed a frequency independent behaviour, over a wide range of frequencies, which is more noticeable for the shear treated gels at both concentrations (Figures 2a and 2b). However, at small frequencies,

viscoelastic properties were more dependent on frequency, which was not significant ($p < 0.05$). By increasing the frequency, G' and G'' values reached a plateau and showed the characteristics of a strong and elastic gel (Ikeda and Foegedings, 1999 and Akkermans, *et al.*, 2008).

The plots of G' and G'' measured at a frequency of 1Hz versus shear rate are shown in Figure 3a and 3b for 10 and 20% concentrations, respectively. At a constant frequency, the values of G' and G'' increased significantly by increasing the shear rate. These results also show that the gels obtained at higher shear rate are considerably stronger with higher elastic modulus at two concentrations. Previously, applying high shear rate and temperature led to the unfolding of soy protein and increasing its flow resistance. Therefore, the obtained shear induced soy protein gels showed a shear thickening property (Ker and Chen, 1998).

The effect of mechanical shearing on the microstructure of whey protein gels at concentrations of 10 and 20% are shown in the microscope images in Figures 4 and 5, respectively. The images obtained from light microscopy, using the 10 $\times$ objective lens, revealed that the untreated whey protein gel had a homogeneous structure at different protein concentrations (Figures 4a and 5a).

Figure 4 and 5 show that high shear rates had significant effects on the microstructure of the gels and shearing created phase separation and generated some aggregations in the structure of the gels. Large pores were observed in the highly sheared gel networks, especially at higher concentration (figures 5d and 5e). However, based on the rheological results, it appears that shearing forms stronger networks and cross linking between the particles. Although phase separation was increased and the structures of the gels were more heterogeneous under shear, the network density changed and the phase separation was not enough to impact the mechanical properties and did not reduce the modulus. Furthermore, in preparation of whey protein gels, the temperature of the whey protein solutions was elevated to 90°C before exposing to different shear rates. Therefore, as described by Moakes *et al.* (2015) and Ikeda and Nishinari (2001), applying high temperature during sol-gel transitions results in the rapidly formed aggregates through covalent bonding. These frigidly structured gels showed more resistant to shear breakdown. According to the theory of rubber elasticity, shear modulus depends on the number of cross links and/or entanglements per unit volume. Therefore, the increase in the elastic modulus can be attributed to an overall increase in the density of cross links in the system (Hosford, 2005).

Formation of a globular protein gel consists of two steps. At the first step, the native protein is partially or totally unfolded through denaturation, while during the second step, the

denatured protein molecules aggregate by covalent (disulphide) and non-covalent (hydrophobic and hydrogen) interactions and three-dimensional protein networks are formed. The solution conditions such as pH, shear and temperature govern both the kinetics of the gelation process and the structure and properties of the gels formed. At neutral or alkaline pH values, covalent disulphide bonds are predominantly responsible for the aggregation of whey protein (Lupano, 2000, Hoffman and van Mill, 1997, Spiegel and Huss, 2002). At these pH range far from isoelectric point, where electrostatic repulsive forces among protein molecules are considerable, more linear aggregates are formed leading to the formation of fine-stranded protein networks (see figures 4a and 5a) (Spiegel and Huss, 2002, Ikeda and Foegedings, 1999). However, the structure and the behaviour of the shear treated gels during heating depend on a balance between aggregation growth and break-up under shear influence. It was shown that applying shearing before heat treatment accelerated protein unfolding and aggregations during heating, hence, the produced gels were stronger (Ker and Toledo, 1992). Furthermore, the response of gels to the shear field during the formation process depends on the temperature of the solution. It is assumed that shear can prevent the formation of the fine-stranded protein networks and promote the process of microparticulation and formation of individual protein aggregates or microgels (Spiegel, 1999). However, applying simultaneous shear and heating at 90°C (above gelling temperature) resulted in the formation of large aggregates with a high G' value compared to that of unsheared gels. By holding at sufficiently high temperature, shear can postpone the gel point leading to the formation of large aggregates (Walkenstrom, *et al.*, 1998). It was showed that by heating whey protein solution between 85 and 95°C at native pH of 6.7, compact shear-induced aggregates were formed and aggregate strands became thick and rigid (Spiegel, 1999). In other words, at neutral pH range, the aggregates grew steadily under shear during heating at high temperature and the degree of denaturation increased (Steventon, *et al.*, 1994, Spiegel, 1999). In the previous researches, whey protein suspensions were exposed to steady shear during heating to the temperatures below 90°C, thus, as a result of significant aggregate break-up under shear, the formed shear treated gels became less elastic than the unsheared gels (Moakes, *et al.*, 2015, Walkenstrom and Hermansson, 1998). However, in our work, applying simultaneous shearing and prolonged heating at 90°C accelerated the aggregation rates of proteins followed by enhancing the formation of the elastic protein network over cooling. During cooling step, the G' values increased as a result of the formation of intermolecular bonds such as hydrogen and hydrophobic bonding. Hence as a result of structural development, the gels became more elastic (Ramos, *et al.*, 2012). Applying prolonged high

temperature led to the rapid formation of firmly-structured gels with covalent bonds and even though the protein solution was under shearing, more particle-particle interactions were favoured.

At lower shear rates of 50 and 100 s⁻¹, whey protein gels become denser and more compact compared to the unsheared gels at two concentrations measured (Figures 4b, 4c and 5b, 5c). However, increasing the shear rate shows an increasing impact of shear on the structure of the gels formed. The heat-induced whey protein gels under two shear rates of 200 and 500 s⁻¹ showed irregular networks, especially at higher protein concentration (See figures 4d, 4e and 5d, 5e). A dense structure was produced by high shear exposure and the network structure became heterogeneous, consisting of compact structures with different large pores. It was suggested that highly structured gels disturbed by shearing can recover immediately and the long-time heating led to restructuring of the gel network (Ikeda and Nishinari, 2001). Therefore, although the microstructure of the gels at higher shear rates becomes more heterogeneous, the elasticity of the gels increases suggesting that stronger gels produced under these highly sheared conditions must contain more effective cross links.

Conclusion

The ability to modify the rheology using shear exposure is important. It also has implications for general processing where the protein systems may be pumped or mixed. It has been shown that shearing had a great impact on the viscoelastic properties of the whey protein gels. Shear treated gels obtained at high temperature and native pH of whey protein were remarkably stronger and more elastic than unsheared gels. At different concentrations, by increasing the shear rate, the moduli increased significantly and exhibited a more frequency independent behaviour. On the whole, shear treated whey protein gels showed progressively more solid like structure. Storage modulus for all the gels reached a steady value at 20°C, showing that the formation of elastic structure developed during cooling and the gels obtained had a stable structure. Images obtained from the gels at different concentrations showed that shearing made some aggregations in the structure of the gels. Also, the phase separation was insufficient to reduce the modulus of the gels. It can be concluded that prolonged heating can result in restructuring of the gel network and shearing at high temperature in the vicinity of neutral pH may form stronger networks and cross linking between the particles.

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Legends to Figures

Figure1a- Storage modulus of 10% whey protein gels at different pre-shear rates obtained over cooling from 90°C to 20°C. Numbers are the mean± standard errors of three replications.

Figure1b: Storage modulus of 20% whey protein gels at different pre-shear rates obtained over cooling from 90°C to 20°C. Numbers are the mean± standard errors of three replications.

Figure2a: Frequency spectrum of 10% whey protein gels at different pre-shear rates obtained at 20°C. Numbers are the mean± standard errors of three replications. Error bars have not been included to avoid cluttering of the graph.

Figure2b: Frequency spectrum of 20% whey protein gels at different pre-shear rates obtained at 20°C. Numbers are the mean± standard errors of three replications. Error bars have not been included to avoid cluttering of the graph.

Figure3a: G' and G'' versus Pre-shear rate obtained at 1Hz and 20°C for 10% whey protein gels. Numbers are the mean± standard errors of three replications.

Figure3b: G' and G'' versus Pre-shear rate obtained at 1Hz and 20°C for 20% whey protein gels. Numbers are the mean± standard errors of three replications.

Figure4: Microstructures of 10% whey protein gels with different shear rates (a) unsheared, (b) 50s⁻¹, (c) 100 s⁻¹, (d) 200 s⁻¹, (e) 500 s⁻¹

Figure5: Microstructures of 20% whey protein gels with different shear rates (a) unsheared, (b) 50s⁻¹, (c) 100 s⁻¹, (d) 200 s⁻¹, (e) 500 s⁻¹

Figure 1a

Figure 1b

Figure 2a

Figure 2b

Figure 3a

Figure 3b

Figure 4a

Figure 4b

Figure 4c

Figure 4d

Figure 4e

Figure 5a

Figure 5b

Figure 5c

Figure 5d

Figure 5e

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