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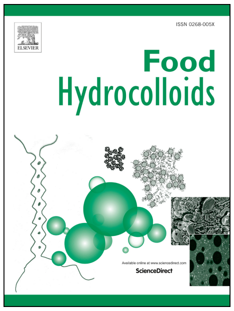

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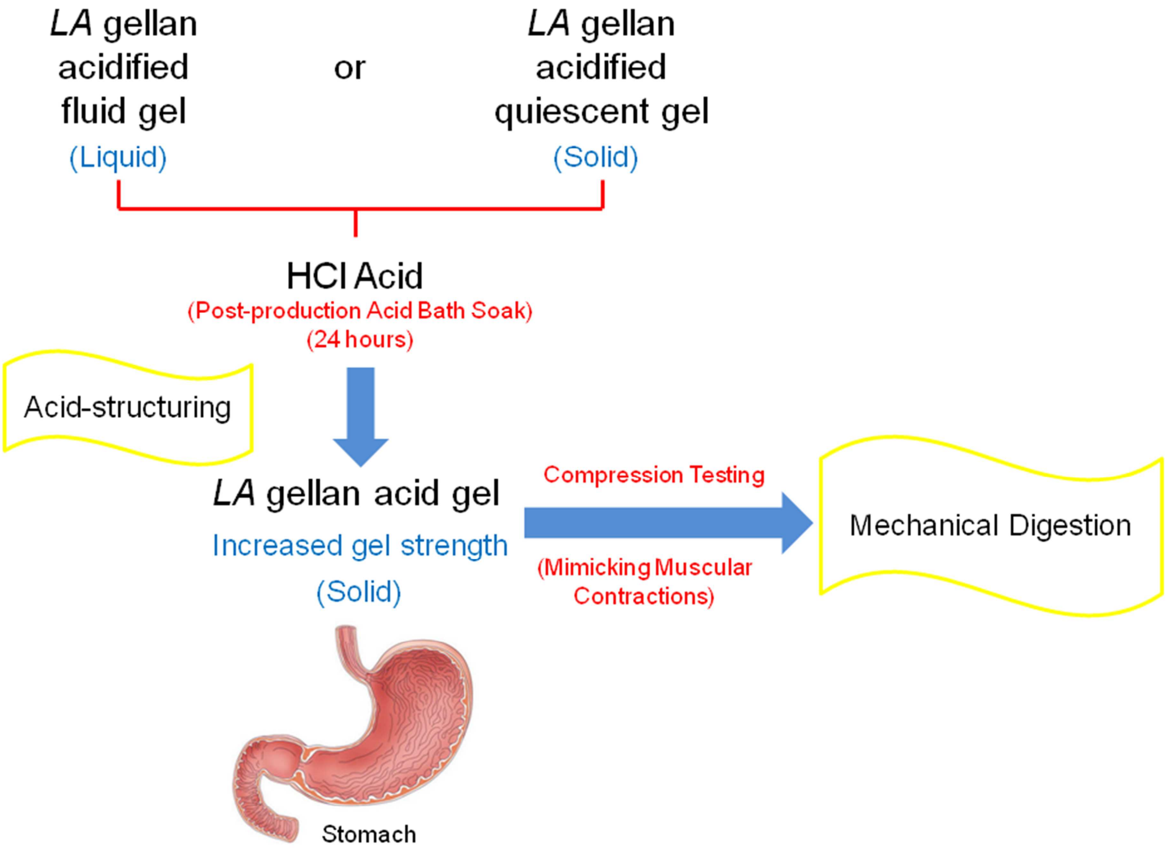
LA gellan acidified fluid gel
(Liquid)
or
LA gellan acidified quiescent gel
(Solid)
HCl Acid
(Post-production Acid Bath Soak)
(24 hours)
Acid-structuring
LA gellan acid gel
Increased gel strength
(Solid)
Compression Testing
(Mimicking Muscular Contractions)
Mechanical Digestion
Stomach

Low acyl gellan gum fluid gel formation and their subsequent response with acid to impact on satiety

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Abstract

Fluid gels have shown potential for use in numerous applications including foods. One such application is in the production of self-structuring food formulations that take advantage of natural digestive processes to increase satiety, potentially helping to combat obesity. The formation and properties of low-acyl gellan gum fluid gels, produced by applying shear during the gelation process are discussed. The acid gelation process of the low-acyl gellan gum fluid gels was investigated through the direct addition of hydrochloric acid, inducing a range of pH environments and also their response to a prolonged exposure to an acidic environment, similar to the conditions found in the stomach. Quiescent *LA* gellan gum gels were also exposed in this way for comparison to the fluid gels.

Rheology was performed on the fluid gels after their formation to determine structure development. Using these methods, the influence of applied shear and acid concentration on the transition temperature, viscosity and molecular ordering in the fluid gel systems has been studied.

Keywords

Fluid gel

Low acyl gellan gum
Process conditions
Material properties
Acid gelation

1. Introduction

Polysaccharides are widely used as replacements for fats and sugars in low fat and reduced calorie foods or within enhanced satiety products (Tang et al., 1994; Brown et al., 1996; Garrec et al., 2012; Norton et al., 2006). The use of hydrocolloids only as simple gelling and thickening agents does not offer any specific benefit to health, convenience or quality in a formulated product (Gabriele et al., 2009). This has led to the development of hydrocolloid fluid gels, which can be designed to achieve a wide range of structural properties (Cassin et al., 2000; Norton et al., 2000) to impart specific advantages to formulated foods.

Fluid gels are formed when shear stress is applied to a solution undergoing gelation; as a result particles are formed within the solution rather than the entire solution forming a homogenous gel. These particles of gel exist as a highly concentrated (high volume fraction) suspension, giving rise to an entirely different set of material properties to the quiescently formed gel (Brown et al., 1996). The application of fluid gels in food formulations has been investigated, and many studies manipulate the ability of some polysaccharides to form solid, brittle gels under quiescent cooling. One such polysaccharide is Gellan. Gellan gum (Sworn et al., 1995; Sworn, 2000; Sworn, 2009; Valli & Miskiel, 2001; Caggioni et al., 2007) is a multifunctional hydrocolloid which is already available for use in a wide variety of food products as a gelling and stabilising agent (Kelco Division of Merck and Co., 1993; Sanderson, 1990; Gibson, 1992). The gellan polymer consists of monosaccharides b-ᴅ-glucose, b-ᴅ-glucuronic acid and α-L-rhamnose in molar ratios of 2:1:1 (Sanderson, 1990) linked together to form a primary linear structure. The biopolymer is produced with two acyl substituents present on the 3-linked glucose, L-glycerol positioned at O(2) and acetyl at O(6). Direct recovery of the polysaccharide from the fermentation broth yields the high acyl form whereas deacylation by alkali treatment results in a low acyl counterpart. Gellan gum is currently commercially available in both the high acyl and the low acyl form. When hot solutions of gellan gum are cooled in the presence of gel-promoting cations, gels ranging in texture from brittle to elastic are formed, principally through cation-mediated helix-helix aggregation (Gibson & Sanderson, 1997). This paper reports results obtained with low acyl gellan gum since it forms clear gels, while high acyl gellan yields opaque gels with less thermal stability. It is important to note however that clarity is not a natural attribute of low acyl gellan gum, it is the result of an additional clarification process that is not applied to the high acyl gellan gum commercial product. The gel phase transition temperatures of low acyl gellan (30 – 50 °C) are lower than those of high acyl gellan gum (70 – 80 °C), which also makes the formation of fluid gels less complex.

To form low acyl gellan gum fluid gels with deionised water, four steps must be followed: (a) dispersion, (b) hydration, (c) addition of either salt or acid and (d) the cooling process, which leads to a quick set as soon as the gelling temperature is reached (Valli et al., 2010). The traditional mechanism proposed for the sol-gel transition of gellan gum is based on a random coil to double helix transition followed by helix to helix aggregation, which involves weak interactions such as Van der Waals forces and Hydrogen bonds. Gel-promoting ions reduce the effect of electrostatic repulsions among helices, augmenting the development of a network. In addition, ionic bridges among carboxylic groups of neighbouring chains have been reported to occur when divalent cations are used (Tang et al., 1997; Nickerson et al., 2003). The properties of gellan gum gels, such as texture, setting temperature and melting temperature, are dependent upon the types of ions present and their concentrations (Sanderson et al., 1988).

The ordering of helices in the production of gellan gum fluid gels is restricted by the shear force applied to small volumes during the cooling step (Valli & Miskiel, 2001), the greater the shear, the smaller the volumes in which ordering can occur, the smaller the resultant fluid gel particles. Weak gels with a similar consistency to fluid gels have also been produced, by allowing gelation to occur in quiescent conditions, provided specific gellan and gel-promoting ion concentrations are used (Sworn et al., 1995; Rodríguez-Hernández et al., 2003). These are structurally different however, as Rodríguez-Hernández et al. (2003) visualised the heterogeneous microstructure of Na-low acyl gellan gum weak gels by confocal scanning laser microscopy (CSLM), showing a network structure rather than the particulate structure of a fluid gel.

Within the food industry, gellan gum fluid gels have numerous applications as suspension and emulsion stabilisers, working at lower concentrations than standard thickeners (García et al., 2011). They also find uses in paint formulations, ophthalmic drug delivery systems (Suri et al., 2006; Sultana et al., 2006), and in medicine for the preparation of artificial lacrimal fluids (García et al., 2011). Gellan gum fluid gels exhibit highly pseudoplastic flow, which provides stable suspension of large included particles combined with low viscosity at higher rates of shear. When used in foods, this results in low viscosity in the mouth, so they can be used to great effect in beverages because of the low viscosity under shear, whilst still allowing for suspension of fruit pulp or jelly pieces. This suspension can be achieved without adversely affecting mouthfeel.

In this work, we aim to investigate the influence of processing conditions during low acyl gellan gum fluid gel formation using two common production methods, and determine how an acid environment affects the structural properties of the resultant fluid gels. This will enable improved understanding of their structure and material responses for their potential application in self-structuring satiety improving food products.

2. Materials and methods

2.1. Sample preparation

Low acyl (*LA*) gellan gum (Kelcogel F, CPKelco, UK) was used to prepare the model acid-sensitive hydrocolloid system in this study, with no further purification or modification to its properties. The water used for the prepared hydrocolloid solutions was passed through a reverse osmosis unit and then a milli-Q water system. Hydrochloric acid was purchased from Fisher Scientific (Loughborough, UK) and was used for the acid bath soaks and direct acidification of the fluid gels.

2.2 Fluid gel production

Fluid gel production and rheological measurements (both during and after fluid gel production) were carried out in a Gemini HR nano stress-controlled rheometer using a 4° truncated (150 μm) cone (40 mm in diameter) and plate geometry. This approach allows for the precise control of the temperature, rate of cooling and the applied rate of shear, which determine the structure of the produced fluid gels. Solutions of 2 wt. % *LA* gellan gum were transferred via pipette to the rheometer plate at 90 °C. The sample temperature was allowed to reach equilibrium (2 minutes) after the geometry was lowered and excess material removed. All experiments were carried out using a thin film of silicon oil, around the outer edge of the geometry, to control evaporation. Cooling was then performed whilst a constant shear between 50 - 1000 s^{-1} was applied, with a cooling at a rate of 3 °C/min. By conducting these sheared cooling profiles with *LA* gellan gum solutions, fluid gels are formed and their viscosities during formation can be measured. Note that due to the cooling constraints of the rheometer equipment, the maximum cooling rate possible is 6 °C/min.

A pin-stirrer was used to produce the *LA* gellan gum fluid gels on a larger scale than the rheometer method. The pin-stirrer consists of a rotating shaft (13 mm diameter) with 8 pairs of pins evenly distributed along its length, which is inserted into a jacketed vessel with 8 pairs of stator pins along the length of the inside wall. The rotating pins pass between the stator pins as they rotate, increasing the anisotropy of the flow field. The average shear rate within the pin stirrer apparatus has been shown to be about 50 s^{-1} with these process conditions (Gabriele, 2011). The hot *LA* gellan gum solution was fed into the pin-stirrer. The internal pin-stirrer volume (with shaft inserted) was approximately 140 ml. The temperature of the fluid entering (T_{in}) and exiting (T_{exit}) the pin-stirrer were recorded using thermocouples, and T_{exit} was controlled via a recirculating water bath that ran through the cooling jacket of the pin-stirrer. Silicon tubing was used to connect all of the units within the process, and material flow was induced via a peristaltic pump (Masterflex® L/S®). A shaft rotation speed of 1500 rpm was used, with the flow rate maintained at 100 ml/min. Samples were then collected, refrigerated (3 °C) and stored for at least 24 h, before testing, to allow post processing ripening effects to fully take place (Gabriele et al., 2009).

2.3 Direct and post-production exposure to acidic environment

Both *LA* gellan gum fluid and acid fluid gels were assessed post-production in terms of their response to a prolonged exposure to an acidic environment that simulates the conditions found in the stomach during digestion. Quiescent *LA* gellan gum gels were also exposed in this way for comparison. The fluid gels (1 wt. %) were produced using the jacketed pin-stirrer and were placed into visking dialysis tubing (23mm ID, 14 kDa pore size, Medicell International, Ltd.) immediately after production, which was then allowed to soak in a 0.5 % HCl acid solution (corresponding to ~ pH 1) for 24 hours. The acid soaking time of 24 hours was hugely exaggerated to take into consideration exposure extremities and the possible effects of chemical breakdown. Texture analysis of these systems was then performed to assess their structural properties. Acid exposure (after approximately 1 hour of soaking) caused the gel strength of the samples to increase and for them to change state from a fluid to a solid. The strengths of the solid gel samples formed enabled compression testing to be performed. After 24 hours of soaking no obvious chemical breakdown of the systems during prolonged acid exposure was observed, with all samples remaining to fit the requirements for compression testing. Mechanical breakdown of the systems then proceeded.

2.4. Measurement techniques

2.4.1. Rheology

To investigate the post-production flow behaviour, rheological analysis was performed on the 2 wt. % *LA* gellan gum fluid gels produced by using either the rheometer or pin-stirrer and the 1 wt. % *LA* gellan gum acid fluid gels produced using the pin-stirrer, immediately after their production. The Malvern Gemini HR nano rheometer was used, and the tests were performed at 25 °C for a range of shear rates, frequencies and strains, using a 4°, 40 mm cone and plate geometry for the 2 wt. % *LA* gellan gum fluid gel and a 40 mm parallel plate geometry (1 mm gap size) for the 1 wt. % *LA* gellan gum fluid gel samples.

2.4.2. Texture Analysis

The structures of the prepared *LA* gellan gum fluid and acid fluid gels following post-production HCl acid exposure were assessed by performing a series of compression tests using a TA.XT.plus texture analyser (Stable Micro Systems Ltd., UK), fitted with a 40 mm diameter cylindrical aluminium probe. All samples had a diameter of 23 mm and length of 10 mm. All measurements were carried out in triplicate with a compression rate of 1 mm/s, with all samples being compressed to fracture. Slippage of the gel samples did not pose a problem with the data obtained.

The force/distance (of compression) data, as obtained directly from the texture analyser, was converted into true strain (ε_H) and true stress (σ_T) data to obtain true stress/true strain curves for all of the *LA* gellan gum fluid and acid fluid gel acid structures according to Bradbeer et al. (2014). From the obtained true stress/true strain curves, the slope of the initial linear region (up to strain values of 0.05 %) can be used to calculate the Young's modulus (Smidsrød et al., 1972) while the slope of the second linear region (for strain values over ~ 0.1 %), leading to the subsequent structure failure can be used to calculate the bulk modulus (Nussinovitch, 2004). The calculated moduli provide information regarding the two deformation mechanisms associated with each of the two linear regions. When the samples are initially loaded the connections between the hydrocolloid molecules within the gel network are bent, as a result of the applied stress. During this compression stage the gel matrix exhibits an elastic behaviour, a measure of which is given by the calculated Young's modulus. When a critical stress is reached the connections between the hydrocolloid chains collapse and the process of deformation enters a second much steeper linear region, during which packing of the hydrocolloid chains takes place. During this compression stage the exhibited behaviour is non-elastic and the slope of the linear region in the true stress/true strain curve, thus the calculated bulk modulus, relates to the stiffness/deformability of the gel matrix, until structure failure occurs. Finally the total work of failure (Kaletunc et al., 1991) is the total work (given as work per unit area in this study) that is required in order for the structure to fail and is represented by the area, up to the point of failure, under the true stress/true strain curve.

3. Results and Discussion

3.1. A comparison of the two methods of fluid gel production

3.1.1. Viscometric response

Viscosity profiles on cooling from 90 °C at constant shear rates between 50 - 1000 s^{-1} and 3 °C/min are shown in Fig. 1 in order to investigate the effect of the applied shear on the production of *LA* gellan gum fluid gels. The obtained data across all shear rates shows that as the temperature of the system is lowered, a sharp increase in viscosity occurs at ~ 42 °C. This viscosity increase has been ascribed (Norton et al., 1999) to the formation of small gel nuclei (initiation of ordering), which begins close to the gelation temperature. On continued cooling, the initially formed gel nuclei continue to grow until an equilibrium particle size, as determined by the shear regime, is reached. It is at this point that a fluid gel has been formed.

The initial formation of the small gel nuclei is thought to be the consequence of a demixing process that results in the formation of polymer-rich and polymer-poor regions in the system. Norton et al. (1998) suggested that demixing occurs either via spinodal decomposition or nucleation and growth. Either way, it is apparent that particles start to form in the early stages of the aggregation process, during which the nuclei are subjected to the applied shear forces and will appear to behave as water-in-water emulsion droplets; thus it is expected for both droplet coalescence and droplet break-up phenomena to take place within the system (Gabriele et al., 2009). In terms of the growth of the fluid gel particles, it has been suggested (Norton et al., 1999) that this occurs either via an “enrichment” process, of the initially small nuclei, from the surrounding non-gelled matrix or due to the coalescence/agglomeration of the particles being forced to come together under the applied shear flow. Thus, it is clear that the observed rapid increase in viscosity is a direct result of the continual increase in both the number and volume fraction of the formed particles, which occurs at a temperature range approaching the hydrocolloid’s gelation temperature.

On further cooling past 30 °C (Fig. 1) the rapid increase in viscosity slows, and further increases are much more gradual for all of the applied shear rates. This observed change in viscosity could be a result of further ordering of a small number of remaining disordered polymer chains within the particles and/or at their surface; conformational ordering persists even at temperatures much lower than the gelling temperature. This would be expected to slightly increase the size of the particles and thus the viscosity during their production. Additionally, the observed behaviour could be a consequence of the inter-particle interactions that take place as a result of the presence of disordered charged polymer chains at their surface (Norton et al., 1998) which likely bind to free ions in the surrounding particles, forming inter-particle bridges. As the applied shear rate is increased (for example from 200 s^{-1} to 300 s^{-1}) these chains are drawn towards the fluid gel particles as a consequence of the greater shear-induced fluid flow, forming a much smoother surface and thus limiting the likelihood of any inter-particle interactions. This in turn results in the observed decrease in the final viscosity (the viscosity measured at the end of the fluid gel production process at 5 °C) as a function of the applied shear. Consideration should also be made to the contribution to the increase in viscosity with decreasing temperature, typically known to obey the Arrhenius model (Gabriele et al., 2009). Alternatively, gel shrinkage as each gel particle becomes more ordered structurally could also be responsible for the reduced rapid viscosity increase rate on cooling past 30 °C.

The viscosity of fluid gel samples (all 2 wt. %), produced using a range of applied shear rates (50 – 1000 s^{-1}) and at a constant cooling rate of 3 °C/min, was measured immediately after the end of their formation process; data for 50 s^{-1} is shown on a log-log scale in Fig. 2. After production, all samples (shear rates 50 – 1000 s^{-1}) exhibited comparable shear thinning (power law) behaviour, despite the assumed differences in particle size between them. Very small differences in the viscosities were observed with increasing shear rate; with a higher shear rate giving rise to smaller values (~ 80 Pas smaller for a shear rate of 1000 s^{-1}). The reason behind this behaviour is that in highly concentrated systems of particles, the flow is characterised on the micro scale by the relative flow of particles "squeezing" past each other. The bulk viscosity would thus be a direct function of the deformability (intrinsic elasticity) and packing of the particles, similarly to the behaviour of highly “packed” emulsions (Mason et al., 1996).

For the fluid gels produced using the pin-stirrer, the applied shear (1500 rpm rotating shaft speed) and cooling rate (30 °C/min; 20 °C at water bath) were kept constant throughout. This high cooling rate that the particles were exposed to within the pin-stirrer makes it very difficult to make a direct comparison of the fluid gel samples formulated using the rheometer production method, due to the cooling constraints of the rheometer equipment (maximum cooling rate = 6 °C/min). Thus, comparisons of the respective fluid gel samples were based solely on their shear rates during production.

The post-production flow behaviour was measured, by recording the viscosity of the fluid gel sample (2 wt. %) 24 hours after production. The data is shown on a log-log scale in Fig. 2, where an almost identical shear-thinning behaviour is observed to that of the fluid gel sample produced using the rheometer at the calculated equivalent shear rate (50 s^{-1}). The conclusion therefore is that the particle sizes are similar independent of the method of production, so long as the shear rate during gelation is comparable.

3.1.2. Material response over time

Fluid gel systems involve inter-particle interactions that persist post-processing, and further develop or ripen for a significant time after processing completion. To understand the time scale over which this strengthening process occurs kinetic studies were implemented. For both the 2 wt. % *LA* gellan gum fluid gels produced using the rheometer and the 2 wt. % *LA* gellan gum fluid gels produced using the pin-stirrer, an oscillatory frequency of 1 Hz and a 1 % strain were applied to the fluid gel samples after production following a 1 hour resting period, and the elastic and viscous modulus responses were measured as a function of time. The results are shown in Fig. 3.

Fig.3 shows that for the fluid gel sample produced using the rheometer the storage modulus remains at a constant level over time. The viscous modulus also remained constant over time, except for a 15 Pa decrease at the start. This could be due to the low shear rate allowing more inter-particle interactions to occur during formation, but it is likely to be a mere measurement error during the time period where the oscillation experiment is first being established. Particularly, since a similar decrease was not observed with the viscous modulus recorded for the fluid gel sample produced using the pin-stirrer (Fig. 3).

In contrast, the data shown in Fig. 3 for the fluid gel sample produced using the pin-stirrer exhibits much higher viscous and elastic moduli, although both remain constant over time. This is accounted for by the high cooling rate (30 °C/min) used with the pin-stirrer method where the gelation process dominates, resulting in the production of fluid gels that have high storage modulus characteristics, and thus more solid-like behaviour.

The results indicate that after a 1 h resting period, both fluid gel sample networks have reached their maximum inter-particle interaction levels, with no further strengthening or ripening occurring thereafter.

Overall, each of the fluid gel samples produced by the two methods display weak gel behaviour. This can be explained structurally; closely packed particle interactions allow an elastic network to form at rest, whilst under shear, they are disrupted allowing material flow.

3.1.3. The coil-helix transition

Low-amplitude oscillation measurements were performed on the fluid gel samples produced using each production method after their formation, during heating and cooling. These enabled the coil-helix transition temperatures to be identified, allowing an improved understanding into the molecular ordering.

Figure 4 shows the elastic and viscous moduli at 1.585 Hz for 2 wt. % low acyl gellan gum fluid gels, produced within a rheometer (50 s^{-1} applied shear rate, 3 °C/min cooling rate) following frequency tables from 0.1 – 10 Hz performed every 10 °C during temperature heating (a) and cooling (b) steps between 20 – 90 °C. On heating, the aggregated regions must be disassembled before the helix-coil transition can occur. This is represented by the steep decrease in the elastic modulus between 20 – 30 °C, with the helix-coil transition occurring thereafter between 30 – 70 °C. Above 70 °C, the solution exists in the disordered coil state. However, a steep increase in the elastic modulus is evident between 80 – 90 °C. The elastic modulus data point at 90 °C is most likely to be a result of sample evaporation, which is possible when performing tests of this type at high temperatures that are close to the boiling point of water, even with the appropriate evaporation prevention methods in place. This leads to an increased concentration of the gellan aqueous solution, forcing the disordered coils closer together causing them to have restricted mobility, and hence increased solid-like character.

The midpoint temperature (T_m) is the midpoint between the two linear regions corresponding to disordered and ordered polymer conformations. For the helix-coil transition in Fig. 4a, $T_m = \sim 50$ °C. T_m represents the temperature at which the number of moles of saccharide residues in the disordered and ordered states are equal, and where the events of helix growth (zipping-up) and decay (un-zipping) are occurring at equal rates (Goodall & Norton, 1987).

During the frequency test on cooling (Fig. 4b), we essentially monitored the formation of a quiescently cooled gel, since the test was performed immediately after melting the fluid gel, and with no external shear force applied, from 90 – 20 °. As with heating, three distinct conformational regions exist in the elastic modulus data: 90 – 60 °C, the solution exists in the disordered state; 60 – 40 °C, the coil-helix transition occurs; 40 – 20 °C, formation of an ordered structure via aggregation. For the coil-helix transition, the $T_m = \sim 50$ °C. The coil-helix transition has been described as a dynamic equilibrium where the rate constant at T_m is zero, and on decreasing temperature the likelihood of growth events is increased relative to those of decay (Norton et al., 1983). Thus molecular rearrangements to both quiescent and fluid gels, via decay and growth mechanisms are reduced on decreasing temperature below T_m. The growth mechanism that is most likely to be responsible for the formation of the quiescent *LA* gellan gel in Fig. 4b, involves intermolecular association of the polymer molecules in the solution during the coil-helix transition, which result in the formation of small, soluble clusters of chains. As the extent of association increases (as the temperature is reduced) these clusters grow, until ultimately they interlink to span the entire volume of the solution and form a continuous crosslinked network (Morris et al., 2012).

For both Figs. 4a and b, the elastic modulus values across the temperature range are greater than those recorded for the viscous modulus from the point of gel ordering completion to the region where the solution is first in the disordered coil state. During this latter region, the values follow identical pathways. Interestingly, the elastic modulus values reported for the formation of the quiescent gel are of a higher magnitude (~ 5 times), than those for the melting of the fluid gel. In the absence of shear, the re-ordering during cooling can take place between particles (as well as within particles) i.e. interparticulate helices form, thus the products require a greater force to allow the particles to move past one another and store more energy (greater elastic modulus) (Garrec & Norton, 2012).

It has been suggested that the *LA* gellan gum fluid gel particles consist of an internal polymeric network, and therefore the elasticity of individual particles is equivalent to that of the quiescently cooled gel (Caggioni et al., 2007). The fact that very similar viscoelastic responses with temperature were observed for the fluid gel produced under shear using the rheometer, and with the quiescent gel formed on cooling in the absence of shear (Figs. 4a and b) suggests that this is correct.

The fluid gel sample produced using the jacketed pin-stirrer exhibited similar rheology with temperature on heating and cooling to that formed using the rheometer. On heating and cooling, the three distinct conformational regions were also observed in the elastic modulus data, and as expected the temperature ranges for each were identical for both experiments. These were: 20 – 30 °C, dissembling of the aggregated stable structures (heating) and formation of an ordered structure (cooling); 30 – 50 °C, helix-coil transition (heating) and coil-helix transition (cooling); 50 – 90 °C, the solution exists in the disordered coil state. For each system (fluid and quiescent respectively) the mid-point temperature was $T_m = \sim 40$ °C. The 10 °C difference in this mid-point temperature value compared with that observed for the gels produced using the rheometer method, is accounted for by the thermal lag in the oscillatory measurements due to the mass of the geometry used. However, overall these values together with the helix-coil and coil-helix transition ranges recorded for each fluid gel production method agree well with the gel phase transition temperatures reported in the literature for *LA* gellan gum (García et al., 2011). They also reinforce the ordering initiation temperature of ~ 42 °C from the viscosity measurements during *LA* gellan gum fluid gel formation within the rheometer (Figure 1).

The fluid gels formed using the jacketed pin-stirrer also had elastic modulus values across the temperature range that were greater than those recorded for viscous modulus. As described for the former production method, the elastic modulus values reported for the quiescent gel using this method were also of a higher magnitude than those for the melting of the fluid gel. Additionally, the viscoelastic measurements obtained using this method were found to be approximately 3 times larger than those obtained when using the rheometer to produce a fluid gel. This can be explained through the fact that the fluid gel sample formed using the jacketed pin-stirrer was allowed to rest for 24 hours after production, prior to rheological testing where this is not possible when the fluid gel is made directly on the rheometer itself.

For each production method, the fluid gel samples displayed similar viscous modulus measurements. The fluid gels formulated using the jacketed pin-stirrer displayed on melting viscous modulus values almost identical to those of the elastic moduli up until the end of the helix-coil transition at 50 °C. Whilst, the fluid gels formulated using the rheometer displayed on cooling viscous modulus values almost identical to those of the elastic moduli up until the start of the coil-helix transition at 60 °C. Thereafter, a steady increase was observed, which is reflective of the samples' elastic distortion of the disentangled/entangled network during structural rearrangement, in response to the low-amplitude oscillatory deformation perturbation (Morris et al., 2012).

3.2. Texture analysis and post-production exposure to acidic environment

The previous section compared the two methods of fluid gel production via the use of a rheometer and a jacketed pin-stirrer. The rheometer is advantageous in that it provides a constant and near uniform shear field, with a high degree of control. However, the technique is not particularly typical of industrial processes. The jacketed pin-stirrer method on the other hand, is more representative for commercial processes, thus this was the method chosen for the direct and post-production acid exposure experiments.

Post-production exposure of the *LA* gellan gum fluid gels to an acidic environment can be used to assess their response to prolonged acid conditions that are similar to those found in the stomach during digestion. It is widely accepted (Yamamoto & Cunha, 2007; Norton et al., 2011) that *LA* gellan is acid-sensitive. Research (Norton et al., 2011; Bradbeer et al., 2014) has shown that *LA* gellan gum gel strength increases on exposure to an HCl acid soak. This is a result of the existing gel networks already partially formed within the gels prior to soaking being reinforced on acid exposure. Ordering and aggregation between individual hydrocolloid chains occurs immediately upon acidification (Moritaka et al., 1995). An increase in gel strength was observed qualitively with the 1 wt. % *LA* gellan gum fluid gels (1500 rpm shaft speed; 100 ml/min pump rate; 20 °C water bath) post HCl acid bath soak, by the change in gel state from a fluid to a solid. Fig. 5 shows the resulting true stress/true strain curves following compression tests performed on these solid gels, as a function of the number of runs that the gel was processed through the pin-stirrer unit during fluid gel formation. Each of the samples displayed purely brittle fracture behaviour, with a rapid decrease in the applied stress once the gels fail at strains between 40 – 42.5 %, where a clear fracture point is observed.

In terms of gel stiffness and their resistance to fracture after soaking in acid, minimal differences (0.0260 MPa and 0.0250 J/m^2 respectively) were found between the fluid gel samples collected after the first two cycles through the pin-stirrer. However, for the sample collected after three runs, a reduction in gel strength and stiffness (0.5907 MPa) was observed; a smaller force (0.2347 J/m^2) was needed to induce fracture (Fig. 5). This sample was exposed to the shear force and cooling environment within the pin stirrer for a greater time period than the other samples. It has been reported that interparticle interactions diminish with continued cooling and shearing during processing, although some interactions will still persist (Cox et al., 2009). Therefore, it would be expected for the fluid gel structure to be weaker and less responsive to the acid exposure, resulting in a weaker acid gel. In addition, the cooling rate implemented (32 ml/min) during the fluid gel production was fairly high. Generally, during high cooling rates the gelation process dominates and fluid gels are produced that have high storage modulus characteristics, and thus more solid-like behaviour indicating larger and more irregularly shaped particles (Cox et al., 2009), essentially resulting in a chopped quiescent gel rather than a fluid gel. As a result, a heterogeneous fluid gel sample is produced, consisting of fluid gel particles along with broken-up fragments, which ultimately leads to a weaker gel with a reduced packing fraction. This together with the diminished interparticle interactions within the gel structures reduces the subsequent response to the acid exposure, explaining why the weak gel behaviour is observed.

3.3. Direct exposure to acidic environment

A variety of acid fluid gel structures can be generated through the direct addition (dropwise) of 0.5 % HCl acid to the aqueous *LA* gellan solution at natural pH, during the production process prior to shearing inducing a range of pH environments. However, the rate of aggregation using this direct HCl addition method is expected to be much higher than the rate achieved by thermally, or ionically induced gelation. This suggests that the extent of cross-linking between the polymer chains (and therefore the elasticity and strength of the overall *LA* gellan acid fluid gel structure) during direct addition of HCl becomes less efficient than when cross-linking occurs in thermally or ionically set gels.

Figure 6 shows flow curve data as a function of pH, for 1 wt. % *LA* gellan gum fluid gels produced within the jacketed pin-stirrer (1500 rpm shaft speed, 100 ml/min pump rate, 15 °C water bath), measured 24 hours after production. In terms of viscosity response on pH reduction the acid gels with acidity from natural pH – pH 3 displayed much higher resistances to flow than those at pH 2. Initial acidification from neutral pH to pH 3.5 causes a large increase in ordering and aggregation to occur between the individual hydrocolloid chains immediately upon acidification (Moritaka et al., 1995), resulting in marked increases in viscosity. However, on further decrease in pH below the pK_a of the glucuronate residues of gellan, at ~ pH 3.4 (Haug, 1964), over-structuring occurs, causing the gels at pH 2 to be extremely weak and turbid, and showing precipitation of the polymer (Moritaka et al., 1995). As a result an almost sponge-like, weak structure is created rather than a stronger homogeneous one. It is very likely that gel structuring at pH 2 is disrupted by the shear applied during the acidification process, and that much lower levels of shear-induced disruption would occur for the mixing conditions found in the stomach. Singh (2007) reported that the shear force on the surface of a food particle (~

0.00043 N) is insignificant in comparison to the crushing or grinding force due to the walls of the stomach (0.2 N).

At pH 2, the 1 wt. % *LA* gellan gum acid fluid gel displayed non-Newtonian shear-thinning flow behaviour i.e. the fluids decrease in viscosity with increasing shear stress (Fig. 6). However, for the respective gels with acidities from natural pH – pH3, a range of individual viscosity yield stresses appear to exist between the approximate shear stress range of 0.07 – 1 Pa. For each pH, a minimum force exertion (shear stress) was required to induce flow behaviour. As soon as the yield stresses had been exceeded, the liquid samples then proceeded to display shear-thinning behaviour (Cui, 2004). This pseudoplastic flow behaviour is characteristic of the stiff polysaccharide chains present in gels of this type under such reduced pH conditions (Cui, 2004).

Phase separation is observed as a decrease in the elastic moduli values at 1.00 Hz in Fig. 7 for the 1 wt. % *LA* gellan gum fluid gels produced within the jacketed pin-stirrer (1500 rpm shaft speed, 100 ml/min pump rate, 15 °C water bath) at pH 2, confirmed by the observation of turbidity at the pH level where their strength began to decrease.

The elastic modulus data, which was measured during a temperature ramp (10 – 90 °C), shows (Fig. 7) that, for the remaining pH values, the elasticity and strength of the gels increases systematically with pH reduction. This demonstrates that the gels become stronger, as a result of greater numbers of cross-links between the polymer chains promoted at the lower pH conditions, and is in good agreement with the flow curve data reported in Fig. 6. Albeit in Fig. 6, the pH 4 acid fluid gel displays the greatest strength amongst the gels, compared to the pH 3 acid fluid gels in Fig. 7.

The elastic modulus data (Fig. 7) shows that with decreasing pH gelation occurs sooner. This is observed as the onset of gelation at higher temperatures for the acid fluid gels at lower pH. The elastic moduli recorded for the acid fluid gel samples at acidities of natural pH and pH4 are similar with increasing temperature. Disassembling of the aggregated stable structures occurs, breaking inter-helical bonds etc. (pH 5: 10 – 40 °C; pH 4: 10 – 50 °C), before moving into the conformational helix-coil transition phase (pH 5: 40 – 70 °C; pH 4: 50 – 80 °C), and finally the region where the solution exists in the disordered coil state (pH 5: 70 – 90 °C; pH 4: 80 – 90 °C). The elastic moduli recorded at pH 3 exhibit extended fluid gel deformation events, as a consequence of the speed of the gelation process. Between 10 – 40 °C, a constant elasticity level is observed, which represents a minimum particle size unaffected by cooling or heating below the final gelation set point at 40 °C. During heating between 40 and 70 °C de-fixing of the gelled particle structures proceeds, disordering the hydrocolloid chains within the particles, creating disordered chain segments at the surface of the particle giving them a 'hairy' nature (Cox et al., 2009). The completion of the melting occurs after 90 °C. Between 70 and 90 °C, full conformational disordering and aggregate break up takes place. The absence of a region, where the solution exists in the disordered coil state is explained by the fact that during formation on heating under such low pH conditions, ordering and aggregation between the individual gellan polymer chains occurs immediately upon acidification (Moritaka et al., 1995).

The effects of pH on gellan gelation have been associated with the lower charge density of chains at lower pH values (Horinaka et al., 2004). They suggested that, since the carboxyl group included in the gellan chain is a weak acid group, and that the degree of dissociation of carboxyl groups in aqueous systems is dominated by the dissociation constant, the lower the pH value, the smaller the fraction of dissociated carboxyl groups, making the gellan a less anionic polyelectrolyte. It is then expected that the less anionic chains aggregate with one another more easily, due to the lower electrostatic repulsion. In addition, the decrease in electrostatic repulsion between the intramolecular segments may result in the suppression of gellan chain expansion (Yamamoto & Cunha, 2007), making association even easier. This overall ease of aggregation caused by decreasing the pH via the direct addition of HCl acid explains the decrease in time to reach the gel point, the more densely linked structure at equilibrium and consequent changes in gel strength, deformability and turbidity.

The *LA* gellan gum acidified fluid gels were assessed post-production in terms of their response to a prolonged exposure to an HCl acid environment as described in Section 3.2. Quiescent *LA* gellan gum gels were also exposed in this way for fluid gel comparison. Fig. 8 shows the resulting true stress/true strain curves following compression tests performed on the gel samples, as a function of pH. Each of the samples displayed purely brittle fracture behaviour, with a rapid decrease in the applied stress once the gels fail at strains between 20 - 35 %, where a clear fracture point is observed.

The gelation of gellan can be induced by a reduction in pH, with Grasdalen and Smidsrød (1987) describing HCl acid as “the most potent gel-former”. However, the variation in gel strength with increasing concentration of acid is not monotonic. The addition of acid results in a large increase in break stress (Picone & Cunha, 2011) down to pH 3.5, where it then proceeds to decrease with further pH decline (Norton et al., 2011), until the gels become extremely weak and exhibit precipitation of the polymer by pH 2 (Moritaka et al., 1995). This behaviour is particularly evident in Fig. 8, where the *LA* gellan acid fluid gels at acidities of natural pH were stiffer and more resistant to fracture than those at lower pH levels. In fact, for the samples with acidities of pH 3 and pH 2, the compression tests were unable to be performed, since the gels were too weak to be tested, following their collapse on removal from the visking tubing.

For both the 1 wt. % *LA* gellan gum quiescent and acid fluid gels at acidities of pH natural and pH 4, we observe a reduction in the failure stress and a shift in strain to lower values with decreasing pH. The shift in strain values is generally indicative of an increase in gel brittleness, whilst the reduction in break stress can be attributed to the combination of the acid concentration and low gellan concentration within the sample, giving rise to a weak network.

Minimal differences (0.0565 MPa and 0.0092 J/m^2 respectively) are observed between the total work of failure and bulk modulus data for the *LA* gellan gum quiescent and acid fluid gels at pH 4. However, slightly larger differences (0.0615 MPa and 0.2317 J/m^2 respectively) were observed for the samples at their natural pH, with the quiescent gel sample having both the larger break stress and strain. This observation further reinforces the outcomes of the elasticity data collected for that of the 2 wt. % *LA* gellan gum quiescent and acid fluid gels at their natural pH, in which higher values were reported for the quiescent gel. This can be explained by the theory that in the absence of shear (i.e. for quiescent gels), the re-ordering during cooling can take place between particles (as well as within particles) i.e. interparticulate helices form, thus the products require a greater force to allow the particles to move past one another and store more energy (greater elastic modulus) (Garrec & Norton, 2012). In turn, stronger gels are formed that exhibit greater resistance to deformation past the elastic limit.

4. Conclusions

With the potential for use of fluid gels in low fat and reduced calorie foods, as well as within self-structuring satiety based food formulations that take advantage of the natural digestive processes, their formation and properties were explored.

We have shown that by controlling the material (*LA* gellan gum and HCl acid concentrations) and process (shear and cooling rates during fluid gel production) parameters it is possible to manipulate the properties and size of individual particles as well as the interactions/bridging between them. Both the fluid and acidified fluid gels produced using these production methods displayed non-Newtonian shear-thinning flow behaviour. Very close similarities were observed between the fluid gels produced by both methods, suggesting that the two distinctive processes are capable of producing particle sizes on similar scales.

It was shown through the rheological and texture analysis data that compared with the fluid gels, the quiescent gels were characteristically stronger, due to the formation of interparticulate helices in the absence of shear on cooling. Fluid gels are said to have fewer and shorter helices than quiescently cooled gels due to the disruption of molecular ordering caused by the applied shear during their production (Garrec & Norton, 2012). Despite this, the coil-helix transition and mid-point temperature data recorded for both gel types, fell within the same range (30 – 70 °C), which was agreeable with the literature (García et al., 2011).

Post-production and direct exposure of the fluid gels to an acidic environment resulted in an increase in gel strength. However, the variation in gel strength with increasing concentration of acid (via direct-addition) was not monotonic, with phase separation of the polymer and solvent proceeding for the fluid gel samples with acidities below pH 3. The elastic modulus data for the acidified fluid gels showed that decreasing the pH, leads to a subsequent reduction in the time taken for gelation to occur.

These findings are promising as they clearly demonstrate that the structuring of *LA* gellan gum fluid gels can be controlled by both the process used for their production and by exposure to an acidic environment. The fluid gel remains liquid and therefore has potential for inclusion in the structure of many different types of foods, and yet still structures when exposed to acidic conditions mimicking the stomach environment in the same way as the solid quiescent gel, potentially providing an increase in satiety level.

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Figure Captions

Figure 1. Fluid gel production: viscosity profiles during the sheared cooling of 2 wt. % low acyl gellan gum solutions at 3 °C/min with varying applied shear rates (50 – 1000 s^{-1}).

Figure 2. Shear thinning behaviour of 2 wt. % low acyl gellan gum fluid gels, produced within a rheometer (50 s^{-1} applied shear rate, 3 °C/min cooling rate) and a jacketed pin-stirrer (1500 rpm shaft speed, 100 ml/min pump rate, 20 °C water bath, 30 °C/min cooling rate).

Figure 3. Storage and loss moduli for 2 wt. % low acyl gellan gum fluid gels, produced within a rheometer (50 s^{-1} applied shear rate, 3 °C/min cooling rate) and a jacketed pin-stirrer (1500 rpm shaft speed, 100 ml/min pump rate, 20 °C water bath, 30 °C/min cooling rate).

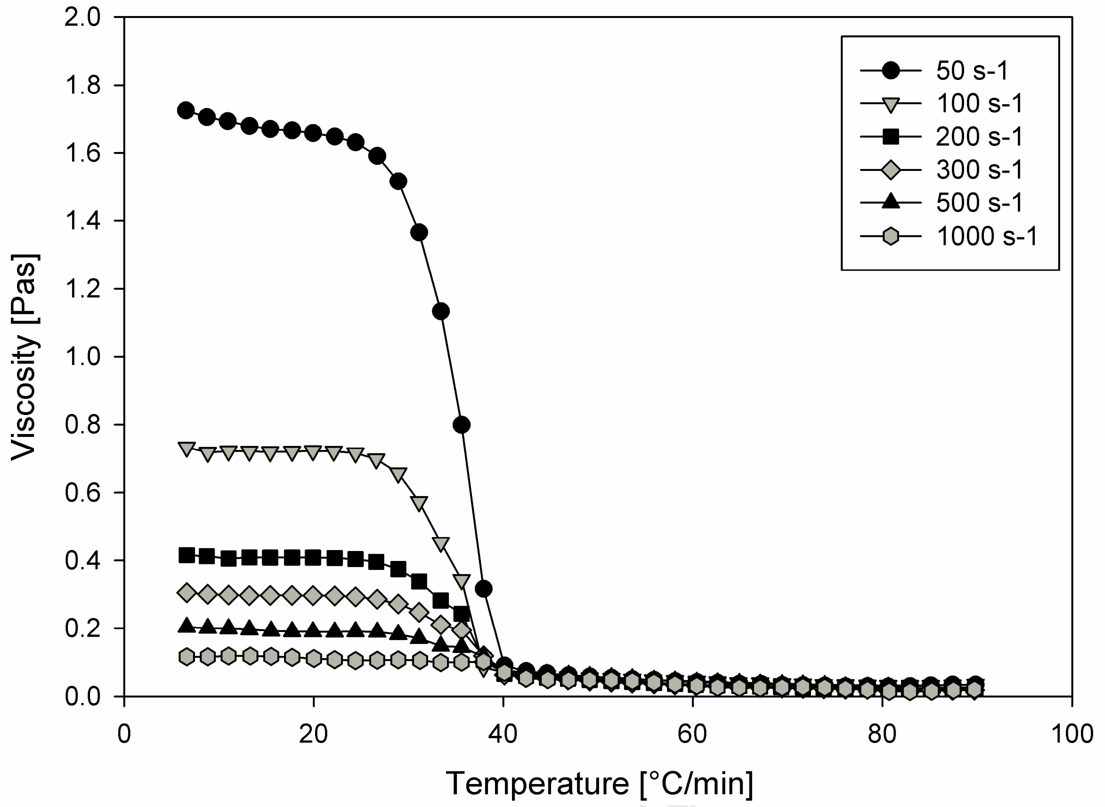
Figure 4. Storage and loss moduli at 1.585 Hz for 2 wt. % low acyl gellan gum fluid gels, produced within a rheometer (50 s^{-1} applied shear rate, 3 °C/min cooling rate) following frequency sweeps (0.1 – 10 Hz) every 10 °C during temperature heating (a) and cooling (b) ramps.

Figure 5. True stress/true strain curves for 1 wt. % low acyl gellan gum fluid gels, produced within a jacketed pin-stirrer (1500 rpm shaft speed, 100 ml/min pump rate, 20 °C water bath, 32 °C/min cooling rate) following exposure to a 0.5 % HCl acid bath soak overnight. All measurements were carried out in triplicate with a compression rate of 1 mm/s. Where error bars cannot be observed, they are smaller than the data points.

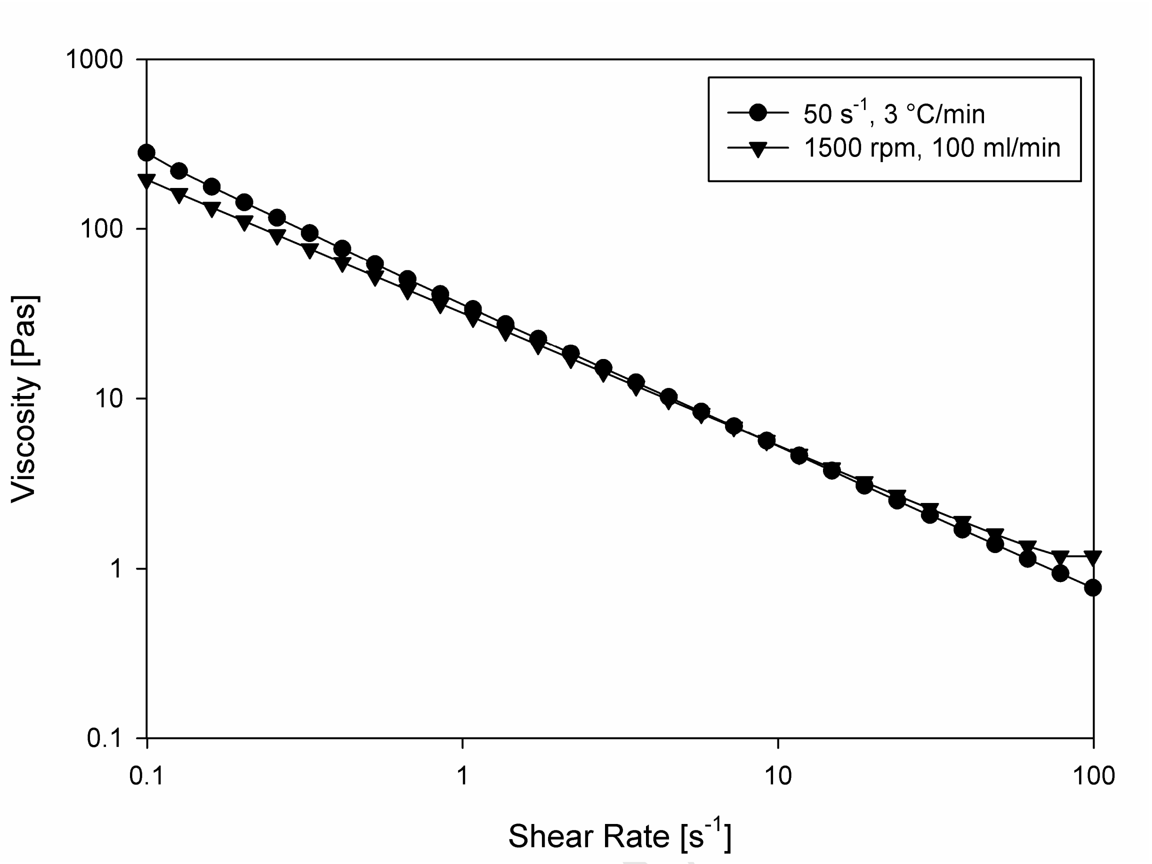
Figure 6. Flow curve data as a function of pH, for 1 wt. % acidified low acyl gellan gum fluid gels, produced within a jacketed pin-stirrer (1500 rpm shaft speed, 100 ml/min pump rate, 15 °C water bath, 32 °C/min cooling rate).

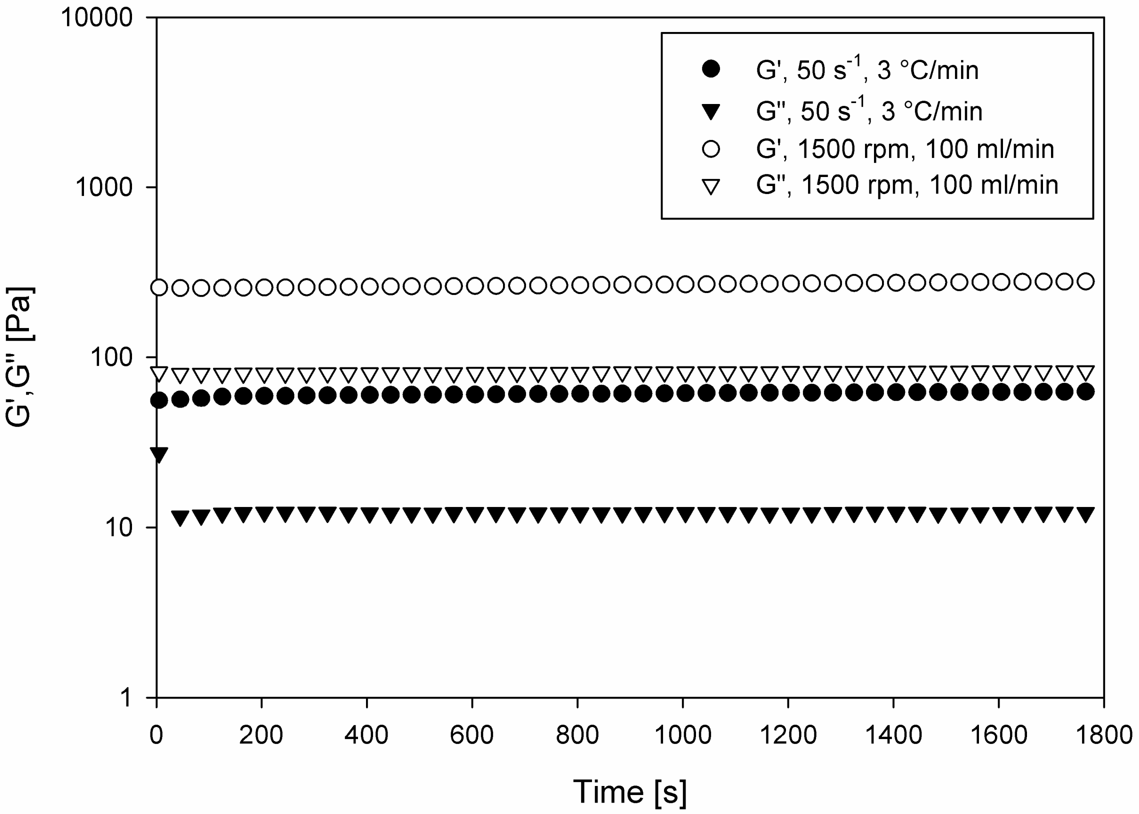
Figure 7. Storage moduli at 1.00 Hz as a function of pH, for 1 wt. % acidified low acyl gellan gum fluid gels, produced within a jacketed pin-stirrer (1500 rpm shaft speed, 100 ml/min pump rate, 15 °C water bath, 32 °C/min cooling rate) following frequency sweeps (0.1 – 10 Hz) every 10 °C during a temperature heating ramp.

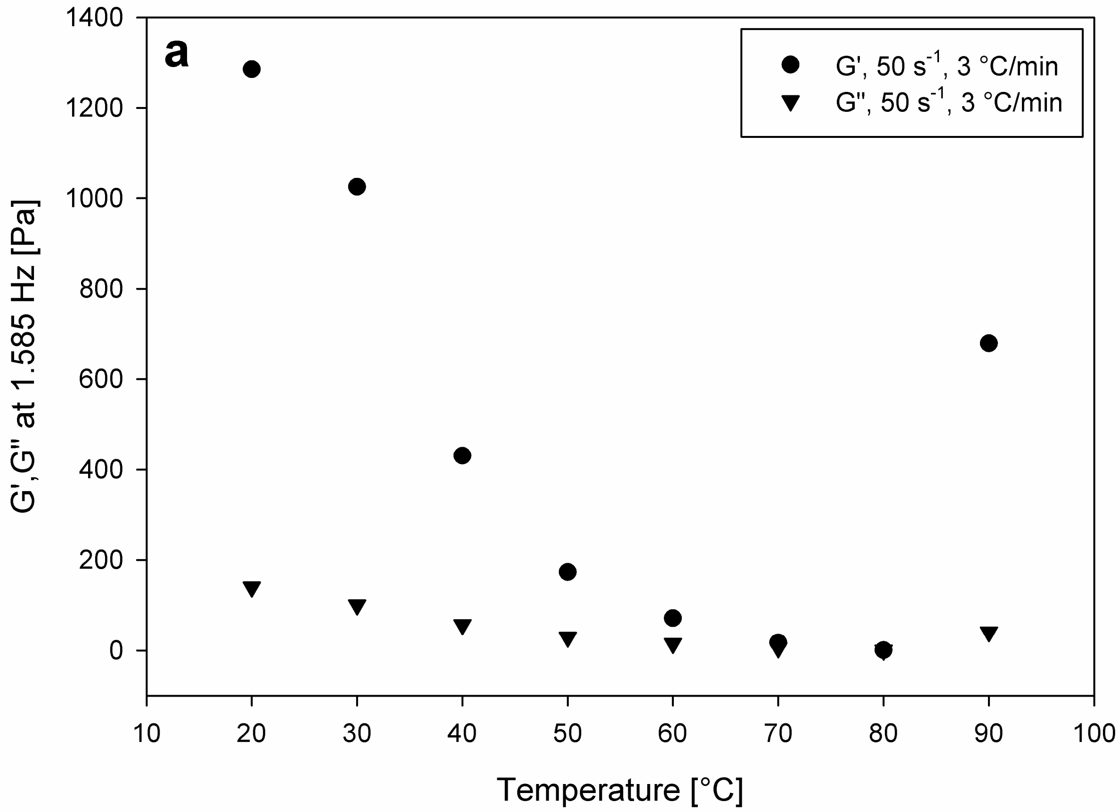
Figure 8. True stress/true strain curves as a function of pH, for 1 wt. % acidified low acyl gellan gum fluid gels, produced within a jacketed pin-stirrer (1500 rpm shaft speed, 100 ml/min pump rate, 15 °C water bath, 32 °C/min cooling rate) and 1 wt. % acidified quiescent gels, following exposure to a 0.5 % HCl acid bath soak overnight. All measurements were carried out in triplicate with a compression rate of 1 mm/s. Where error bars cannot be observed, they are smaller than the data points.

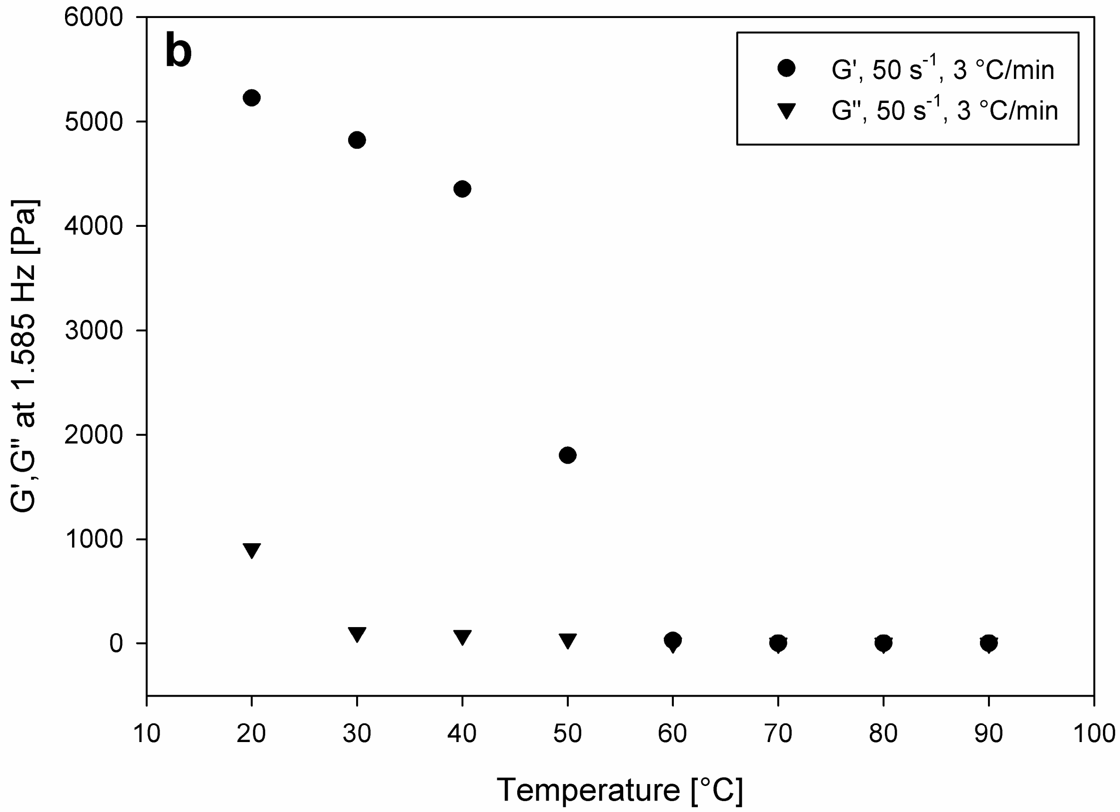


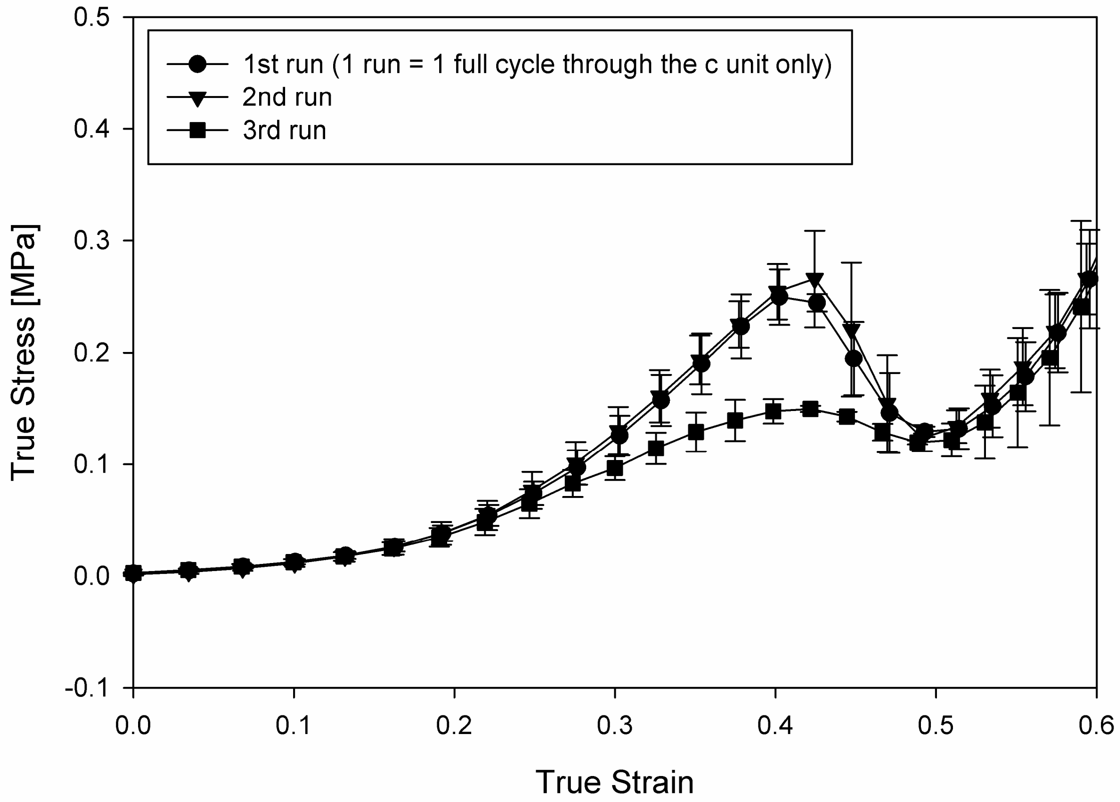
Viscosity [Pas]
Temperature [°C/min]
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
50 s-1
100 s-1
200 s-1
300 s-1
500 s-1
1000 s-1

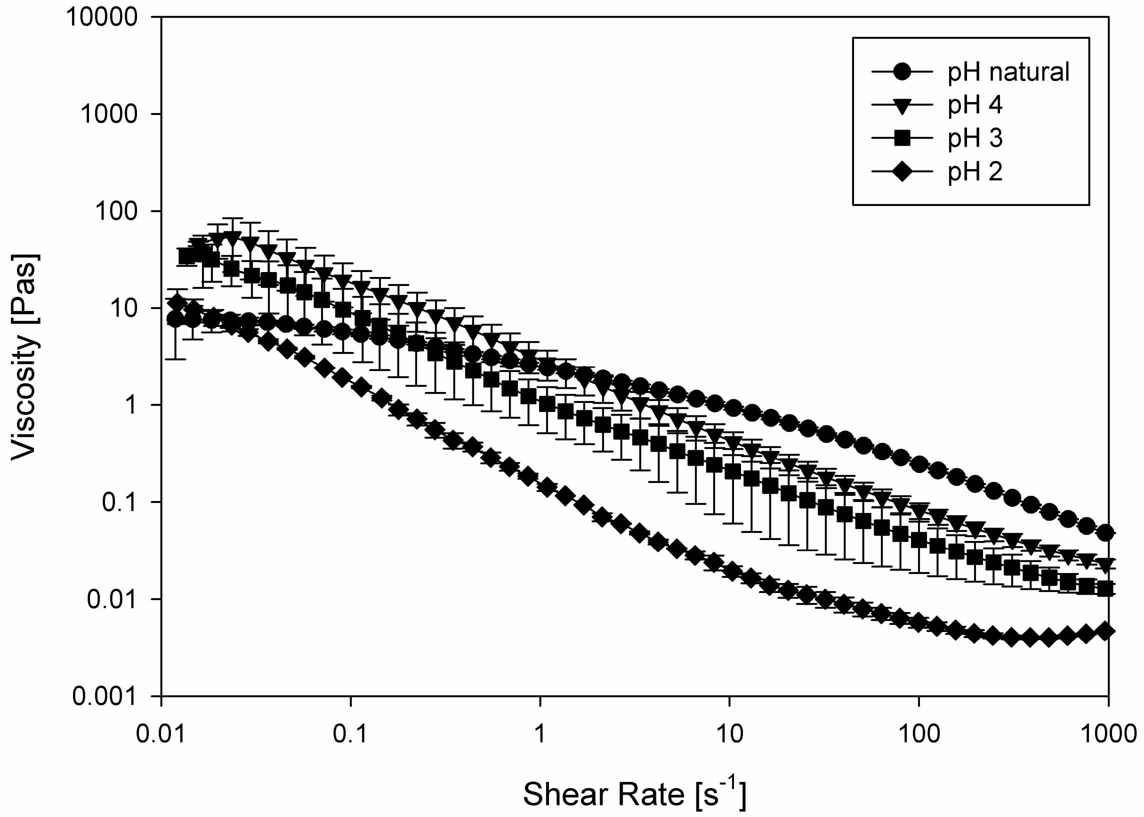

1000
100
10
1
0.1
Viscosity [Pas]
0.1
1
10
100
Shear Rate [s^{-1}]
50 s^{-1}, 3 °C/min
1500 rpm, 100 ml/min

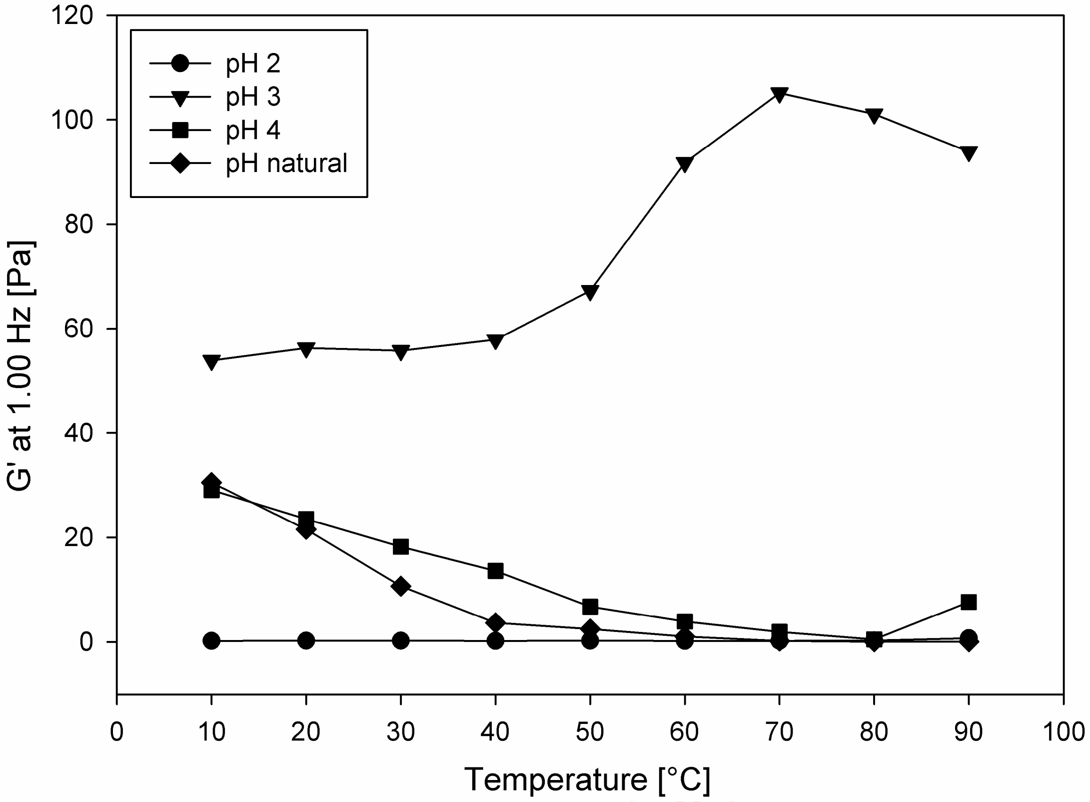

10000
1000
100
10
1
G',G'' [Pa]
0
200
400
600
800
1000
1200
1400
1600
1800
Time [s]
● G', 50 s^{-1}, 3 °C/min
▼ G'', 50 s^{-1}, 3 °C/min
○ G', 1500 rpm, 100 ml/min
▽ G'', 1500 rpm, 100 ml/min

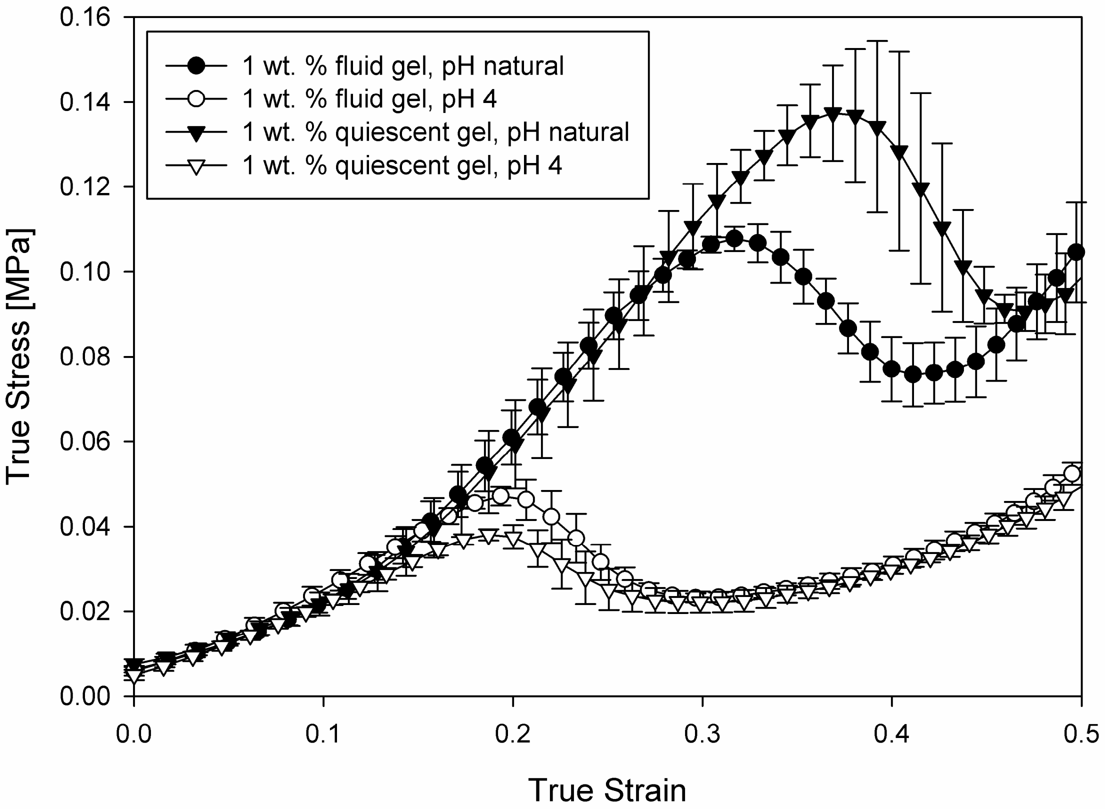

a
G', 50 s-1, 3 °C/min
G", 50 s-1, 3 °C/min
G',G" at 1.585 Hz [Pa]
1400
1200
1000
800
600
400
200
0
Temperature [°C]
10
20
30
40
50
60
70
80
90
100


b
G', 50 s-1, 3 °C/min
G'', 50 s-1, 3 °C/min
G',G'' at 1.585 Hz [Pa]
6000
5000
4000
3000
2000
1000
0
10
20
30
40
50
60
70
80
90
100
Temperature [°C]


0.5
0.4
0.3
0.2
0.1
0.0
-0.1
True Stress [MPa]
1st run (1 run = 1 full cycle through the c unit only)
2nd run
3rd run
0.0
0.1
0.2
0.3
0.4
0.5
0.6
True Strain


10000
1000
100
10
1
0.1
0.01
0.001
Viscosity [Pas]
0.01
0.1
1
10
100
1000
Shear Rate [s^{-1}]
pH natural
pH 4
pH 3
pH 2


pH 2
pH 3
pH 4
pH natural
G' at 1.00 Hz [Pa]
Temperature [°C]
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70
80
90
100


0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
True Stress [MPa]
0.0
0.1
0.2
0.3
0.4
0.5
True Strain
● 1 wt. % fluid gel, pH natural
○ 1 wt. % fluid gel, pH 4
▼ 1 wt. % quiescent gel, pH natural
▽ 1 wt. % quiescent gel, pH 4

Highlights

- Low acyl gellan gum fluid gels were prepared.
- *in vitro* acid-induced gelation of the fluid gel systems was investigated.
- Fluid gel properties are dependent on pH, concentration and processing.
- Post-production exposure to acid increased fluid gel strength markedly.