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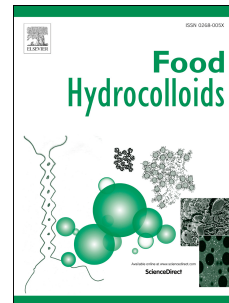
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LA gellan
acidified
fluid gel
(Liquid)

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)

Stomach

Compression Testing
(Mimicking Muscular Contractions)

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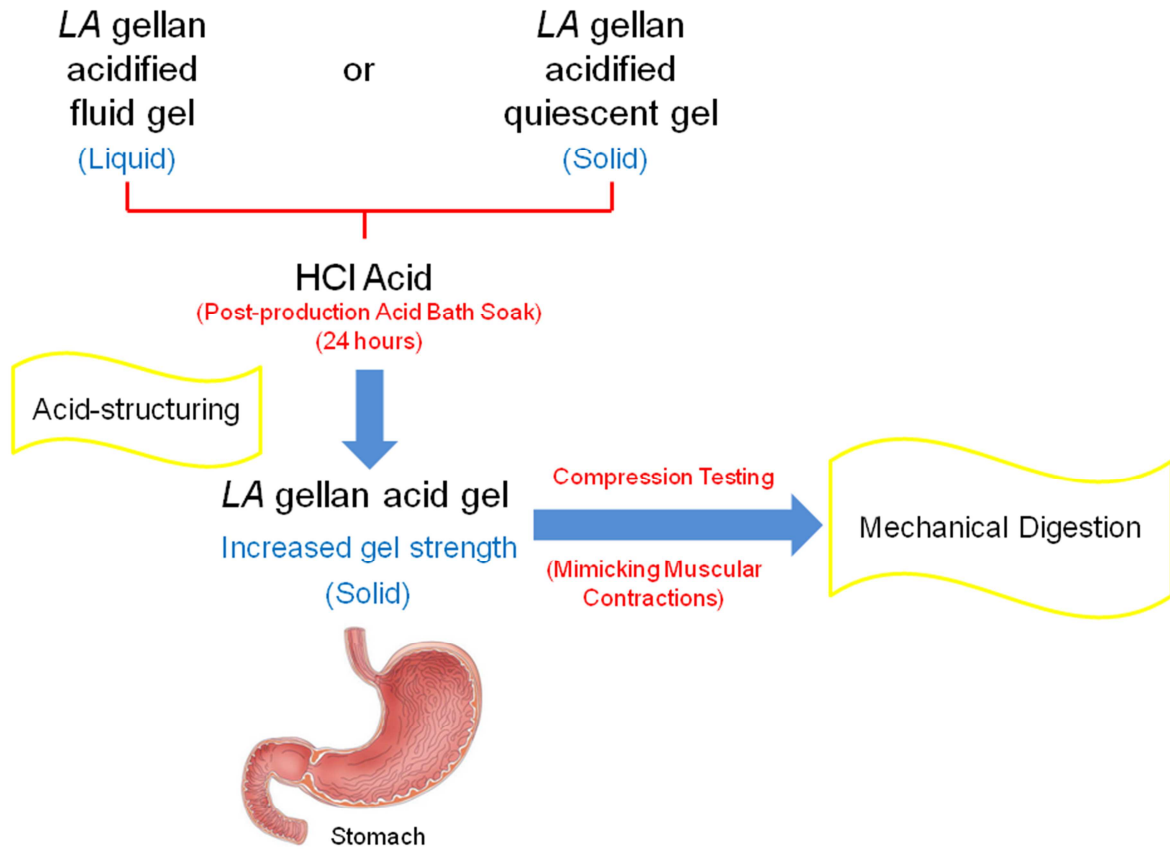
1   Low acyl gellan gum fluid gel formation and their subsequent response with acid to impact on satiety

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7

8   Abstract

9

10   Fluid gels have shown potential for use in numerous applications including foods. One such

11   application is in the production of self-structuring food formulations that take advantage of

12   natural digestive processes to increase satiety, potentially helping to combat obesity. The

13   formation and properties of low-acyl gellan gum fluid gels, produced by applying shear during

14   the gelation process are discussed.   The acid gelation process of the low-acyl gellan gum fluid

15   gels was investigated through the direct addition of hydrochloric acid, inducing a range of pH

16   environments and also their response to a prolonged exposure to an acidic environment, similar

17   to the conditions found in the stomach. Quiescent *LA* gellan gum gels were also exposed in this

18   way for comparison to the fluid gels.

19   Rheology was performed on the fluid gels after their formation to determine structure

20   development. Using these methods, the influence of applied shear and acid concentration on

21   the transition temperature, viscosity and molecular ordering in the fluid gel systems has been

22   studied.

23

24   Keywords

25

26   Fluid gel

27   Low acyl gellan gum

28   Process conditions

29   Material properties

30   Acid gelation

31

32   1. Introduction

33   Polysaccharides are widely used as replacements for fats and sugars in low fat and reduced

34   calorie foods or within enhanced satiety products (Tang et al., 1994; Brown et al., 1996; Garrec

35   et al., 2012; Norton et al., 2006). The use of hydrocolloids only as simple gelling and thickening

36   agents does not offer any specific benefit to health, convenience or quality in a formulated

37   product (Gabriele et al., 2009). This has led to the development of hydrocolloid fluid gels, which

38   can be designed to achieve a wide range of structural properties (Cassin et al., 2000; Norton et

39   al., 2000) to impart specific advantages to formulated foods.

40   Fluid gels are formed when shear stress is applied to a solution undergoing gelation; as a

41   result particles are formed within the solution rather than the entire solution forming a

42   homogenous gel. These particles of gel exist as a highly concentrated (high volume fraction)

43   suspension, giving rise to an entirely different set of material properties to the quiescently

44   formed gel (Brown et al., 1996). The application of fluid gels in food formulations has been

45   investigated, and many studies manipulate the ability of some polysaccharides to form solid,

46   brittle gels under quiescent cooling. One such polysaccharide is Gellan. Gellan gum (Sworn et

47   al., 1995; Sworn, 2000; Sworn, 2009; Valli & Miskiel, 2001; Caggioni et al., 2007) is a

48   multifunctional hydrocolloid which is already available for use in a wide variety of food products

49   as a gelling and stabilising agent (Kelco Division of Merck and Co., 1993; Sanderson, 1990;

50   Gibson, 1992). The gellan polymer consists of monosaccharides b-ᴅ-glucose, b-ᴅ-glucuronic acid

51   and α-L-rhamnose in molar ratios of 2:1:1 (Sanderson, 1990) linked together to form a primary

52 linear structure. The biopolymer is produced with two acyl substituents present on the 3-linked

53 glucose, L-glycerol positioned at O(2) and acetyl at O(6). Direct recovery of the polysaccharide

54 from the fermentation broth yields the high acyl form whereas deacylation by alkali treatment

55 results in a low acyl counterpart. Gellan gum is currently commercially available in both the high

56 acyl and the low acyl form. When hot solutions of gellan gum are cooled in the presence of gel-

57 promoting cations, gels ranging in texture from brittle to elastic are formed, principally through

58 cation-mediated helix-helix aggregation (Gibson & Sanderson, 1997). This paper reports results

59 obtained with low acyl gellan gum since it forms clear gels, while high acyl gellan yields opaque

60 gels with less thermal stability. It is important to note however that clarity is not a natural

61 attribute of low acyl gellan gum, it is the result of an additional clarification process that is not

62 applied to the high acyl gellan gum commercial product. The gel phase transition temperatures

63 of low acyl gellan (30 – 50 °C) are lower than those of high acyl gellan gum (70 – 80 °C), which

64 also makes the formation of fluid gels less complex.

65 To form low acyl gellan gum fluid gels with deionised water, four steps must be followed: (a)

66 dispersion, (b) hydration, (c) addition of either salt or acid and (d) the cooling process, which

67 leads to a quick set as soon as the gelling temperature is reached (Valli et al., 2010). The

68 traditional mechanism proposed for the sol-gel transition of gellan gum is based on a random

69 coil to double helix transition followed by helix to helix aggregation, which involves weak

70 interactions such as Van der Waals forces and Hydrogen bonds. Gel-promoting ions reduce the

71 effect of electrostatic repulsions among helices, augmenting the development of a network. In

72 addition, ionic bridges among carboxylic groups of neighbouring chains have been reported to

73 occur when divalent cations are used (Tang et al., 1997; Nickerson et al., 2003). The properties

74 of gellan gum gels, such as texture, setting temperature and melting temperature, are

75 dependent upon the types of ions present and their concentrations (Sanderson et al., 1988).

76   The ordering of helices in the production of gellan gum fluid gels is restricted by the shear

77   force applied to small volumes during the cooling step (Valli & Miskiel, 2001), the greater the

78   shear, the smaller the volumes in which ordering can occur, the smaller the resultant fluid gel

79   particles. Weak gels with a similar consistency to fluid gels have also been produced, by allowing

80   gelation to occur in quiescent conditions, provided specific gellan and gel-promoting ion

81   concentrations are used (Sworn et al., 1995; Rodríguez-Hernández et al., 2003). These are

82   structurally different however, as Rodríguez-Hernández et al. (2003) visualised the

83   heterogeneous microstructure of Na-low acyl gellan gum weak gels by confocal scanning laser

84   microscopy (CSLM), showing a network structure rather than the particulate structure of a fluid

85   gel.

86   Within the food industry, gellan gum fluid gels have numerous applications as suspension

87   and emulsion stabilisers, working at lower concentrations than standard thickeners (García et

88   al., 2011). They also find uses in paint formulations, ophthalmic drug delivery systems (Suri et

89   al., 2006; Sultana et al., 2006), and in medicine for the preparation of artificial lacrimal fluids

90   (García et al., 2011). Gellan gum fluid gels exhibit highly pseudoplastic flow, which provides

91   stable suspension of large included particles combined with low viscosity at higher rates of

92   shear. When used in foods, this results in low viscosity in the mouth, so they can be used to

93   great effect in beverages because of the low viscosity under shear, whilst still allowing for

94   suspension of fruit pulp or jelly pieces. This suspension can be achieved without adversely

95   affecting mouthfeel.

96   In this work, we aim to investigate the influence of processing conditions during low acyl

97   gellan gum fluid gel formation using two common production methods, and determine how an

98   acid environment affects the structural properties of the resultant fluid gels. This will enable

99   improved understanding of their structure and material responses for their potential application

100   in self-structuring satiety improving food products.

101

102   2.   Materials and methods

103   2.1. Sample preparation

104   Low acyl (*LA*) gellan gum (Kelcogel F, CPKelco, UK) was used to prepare the model acid-

105   sensitive hydrocolloid system in this study, with no further purification or modification to its

106   properties. The water used for the prepared hydrocolloid solutions was passed through a

107   reverse osmosis unit and then a milli-Q water system. Hydrochloric acid was purchased from

108   Fisher Scientific (Loughborough, UK) and was used for the acid bath soaks and direct

109   acidification of the fluid gels.

110

111   2.2   Fluid gel production

112   Fluid gel production and rheological measurements (both during and after fluid gel

113   production) were carried out in a Gemini HR nano stress-controlled rheometer using a 4°

114   truncated (150 µm) cone (40 mm in diameter) and plate geometry. This approach allows for the

115   precise control of the temperature, rate of cooling and the applied rate of shear, which

116   determine the structure of the produced fluid gels. Solutions of 2 wt. % *LA* gellan gum were

117   transferred via pipette to the rheometer plate at 90 °C. The sample temperature was allowed to

118   reach equilibrium (2 minutes) after the geometry was lowered and excess material removed. All

119   experiments were carried out using a thin film of silicon oil, around the outer edge of the

120   geometry, to control evaporation. Cooling was then performed whilst a constant shear between

121   50 - 1000 s^{-1} was applied, with a cooling at a rate of 3 °C/min. By conducting these sheared

122   cooling profiles with *LA* gellan gum solutions, fluid gels are formed and their viscosities during

123   formation can be measured. Note that due to the cooling constraints of the rheometer

124   equipment, the maximum cooling rate possible is 6 °C/min.

125

126   A pin-stirrer was used to produce the *LA* gellan gum fluid gels on a larger scale than the

127   rheometer method. The pin-stirrer consists of a rotating shaft (13 mm diameter) with 8 pairs of

128   pins evenly distributed along its length, which is inserted into a jacketed vessel with 8 pairs of

129   stator pins along the length of the inside wall. The rotating pins pass between the stator pins as

130   they rotate, increasing the anisotropy of the flow field. The average shear rate within the pin

131   stirrer apparatus has been shown to be about 50 s^{-1} with these process conditions (Gabriele,

132   2011). The hot *LA* gellan gum solution was fed into the pin-stirrer. The internal pin-stirrer

133   volume (with shaft inserted) was approximately 140 ml. The temperature of the fluid entering

134   (T_{in}) and exiting (T_{exit}) the pin-stirrer were recorded using thermocouples, and T_{exit} was

135   controlled via a recirculating water bath that ran through the cooling jacket of the pin-stirrer.

136   Silicon tubing was used to connect all of the units within the process, and material flow was

137   induced via a peristaltic pump (Masterflex® L/S®). A shaft rotation speed of 1500 rpm was used,

138   with the flow rate maintained at 100 ml/min. Samples were then collected, refrigerated (3 °C)

139   and stored for at least 24 h, before testing, to allow post processing ripening effects to fully take

140   place (Gabriele et al., 2009).

141

142   2.3   Direct and post-production exposure to acidic environment

143   Both *LA* gellan gum fluid and acid fluid gels were assessed post-production in terms of their

144   response to a prolonged exposure to an acidic environment that simulates the conditions found

145   in the stomach during digestion. Quiescent *LA* gellan gum gels were also exposed in this way for

146   comparison. The fluid gels (1 wt. %) were produced using the jacketed pin-stirrer and were

147   placed into visking dialysis tubing (23mm ID, 14 kDa pore size, Medicell International, Ltd.)

148   immediately after production, which was then allowed to soak in a 0.5 % HCl acid solution

149   (corresponding to ~ pH 1) for 24 hours. The acid soaking time of 24 hours was hugely

150   exaggerated to take into consideration exposure extremities and the possible effects of chemical

151   breakdown. Texture analysis of these systems was then performed to assess their structural

152   properties. Acid exposure (after approximately 1 hour of soaking) caused the gel strength of the

153   samples to increase and for them to change state from a fluid to a solid. The strengths of the

154   solid gel samples formed enabled compression testing to be performed. After 24 hours of

155   soaking no obvious chemical breakdown of the systems during prolonged acid exposure was

156   observed, with all samples remaining to fit the requirements for compression testing.

157   Mechanical breakdown of the systems then proceeded.

158

159   2.4.   Measurement techniques

160   2.4.1.   Rheology

161   To investigate the post-production flow behaviour, rheological analysis was performed on

162   the 2 wt. % *LA* gellan gum fluid gels produced by using either the rheometer or pin-stirrer and

163   the 1 wt. % *LA* gellan gum acid fluid gels produced using the pin-stirrer, immediately after their

164   production. The Malvern Gemini HR nano rheometer was used, and the tests were performed

165   at 25 °C for a range of shear rates, frequencies and strains, using a 4°, 40 mm cone and plate

166   geometry for the 2 wt. % *LA* gellan gum fluid gel and a 40 mm parallel plate geometry (1 mm gap

167   size) for the 1 wt. % *LA* gellan gum fluid gel samples.

168

169   2.4.2.   Texture Analysis

170   The structures of the prepared *LA* gellan gum fluid and acid fluid gels following post-

171   production HCl acid exposure were assessed by performing a series of compression tests using a

172   TA.XT.plus texture analyser (Stable Micro Systems Ltd., UK), fitted with a 40 mm diameter

173   cylindrical aluminium probe. All samples had a diameter of 23 mm and length of 10 mm. All

7

174 measurements were carried out in triplicate with a compression rate of 1 mm/s, with all samples

175 being compressed to fracture. Slippage of the gel samples did not pose a problem with the data

176 obtained.

177 The force/distance (of compression) data, as obtained directly from the texture analyser,

178 was converted into true strain (ε_H) and true stress (σ_T) data to obtain true stress/true strain

179 curves for all of the *LA* gellan gum fluid and acid fluid gel acid structures according to Bradbeer

180 et al. (2014). From the obtained true stress/true strain curves, the slope of the initial linear

181 region (up to strain values of 0.05 %) can be used to calculate the Young's modulus (Smidsrød et

182 al., 1972) while the slope of the second linear region (for strain values over $\sim$ 0.1 %), leading to

183 the subsequent structure failure can be used to calculate the bulk modulus (Nussinovitch, 2004).

184 The calculated moduli provide information regarding the two deformation mechanisms

185 associated with each of the two linear regions. When the samples are initially loaded the

186 connections between the hydrocolloid molecules within the gel network are bent, as a result of

187 the applied stress. During this compression stage the gel matrix exhibits an elastic behaviour, a

188 measure of which is given by the calculated Young's modulus. When a critical stress is reached

189 the connections between the hydrocolloid chains collapse and the process of deformation

190 enters a second much steeper linear region, during which packing of the hydrocolloid chains

191 takes place. During this compression stage the exhibited behaviour is non-elastic and the slope

192 of the linear region in the true stress/true strain curve, thus the calculated bulk modulus, relates

193 to the stiffness/deformability of the gel matrix, until structure failure occurs. Finally the total

194 work of failure (Kaletunc et al., 1991) is the total work (given as work per unit area in this study)

195 that is required in order for the structure to fail and is represented by the area, up to the point

196 of failure, under the true stress/true strain curve.

197

198 3. Results and Discussion

199 3.1. A comparison of the two methods of fluid gel production

8

200   3.1.1.   Viscometric response

201   Viscosity profiles on cooling from 90 °C at constant shear rates between 50 - 1000 s^{-1} and 3

202   °C/min are shown in Fig. 1 in order to investigate the effect of the applied shear on the

203   production of *LA* gellan gum fluid gels. The obtained data across all shear rates shows that as

204   the temperature of the system is lowered, a sharp increase in viscosity occurs at ∼ 42 °C. This

205   viscosity increase has been ascribed (Norton et al., 1999) to the formation of small gel nuclei

206   (initiation of ordering), which begins close to the gelation temperature. On continued cooling,

207   the initially formed gel nuclei continue to grow until an equilibrium particle size, as determined

208   by the shear regime, is reached. It is at this point that a fluid gel has been formed.

209   The initial formation of the small gel nuclei is thought to be the consequence of a demixing

210   process that results in the formation of polymer-rich and polymer-poor regions in the system.

211   Norton et al. (1998) suggested that demixing occurs either via spinodal decomposition or

212   nucleation and growth. Either way, it is apparent that particles start to form in the early stages

213   of the aggregation process, during which the nuclei are subjected to the applied shear forces

214   and will appear to behave as water-in-water emulsion droplets; thus it is expected for both

215   droplet coalescence and droplet break-up phenomena to take place within the system (Gabriele

216   et al., 2009). In terms of the growth of the fluid gel particles, it has been suggested (Norton et

217   al., 1999) that this occurs either via an "enrichment" process, of the initially small nuclei, from

218   the surrounding non-gelled matrix or due to the coalescence/agglomeration of the particles

219   being forced to come together under the applied shear flow. Thus, it is clear that the observed

220   rapid increase in viscosity is a direct result of the continual increase in both the number and

221   volume fraction of the formed particles, which occurs at a temperature range approaching the

222   hydrocolloid's gelation temperature.

223   On further cooling past 30 °C (Fig. 1) the rapid increase in viscosity slows, and further

224   increases are much more gradual for all of the applied shear rates. This observed change in

9

225   viscosity could be a result of further ordering of a small number of remaining disordered

226   polymer chains within the particles and/or at their surface; conformational ordering persists

227   even at temperatures much lower than the gelling temperature. This would be expected to

228   slightly increase the size of the particles and thus the viscosity during their production.

229   Additionally, the observed behaviour could be a consequence of the inter-particle interactions

230   that take place as a result of the presence of disordered charged polymer chains at their surface

231   (Norton et al., 1998) which likely bind to free ions in the surrounding particles, forming inter-

232   particle bridges. As the applied shear rate is increased (for example from 200 s^{-1} to 300 s^{-1})

233   these chains are drawn towards the fluid gel particles as a consequence of the greater shear-

234   induced fluid flow, forming a much smoother surface and thus limiting the likelihood of any

235   inter-particle interactions. This in turn results in the observed decrease in the final viscosity

236   (the viscosity measured at the end of the fluid gel production process at 5 °C) as a function of

237   the applied shear. Consideration should also be made to the contribution to the increase in

238   viscosity with decreasing temperature, typically known to obey the Arrhenius model (Gabriele

239   et al., 2009). Alternatively, gel shrinkage as each gel particle becomes more ordered

240   structurally could also be responsible for the reduced rapid viscosity increase rate on cooling

241   past 30 °C.

242   The viscosity of fluid gel samples (all 2 wt. %), produced using a range of applied shear rates

243   (50 – 1000 s^{-1}) and at a constant cooling rate of 3 °C/min, was measured immediately after the

244   end of their formation process; data for 50 s^{-1} is shown on a log-log scale in Fig. 2. After

245   production, all samples (shear rates 50 – 1000 s^{-1}) exhibited comparable shear thinning (power

246   law) behaviour, despite the assumed differences in particle size between them. Very small

247   differences in the viscosities were observed with increasing shear rate; with a higher shear rate

248   giving rise to smaller values (~ 80 Pas smaller for a shear rate of 1000 s^{-1}). The reason behind

249   this behaviour is that in highly concentrated systems of particles, the flow is characterised on

250   the micro scale by the relative flow of particles "squeezing" past each other. The bulk viscosity

10

251   would thus be a direct function of the deformability (intrinsic elasticity) and packing of the

252   particles, similarly to the behaviour of highly "packed" emulsions (Mason et al., 1996).

253   For the fluid gels produced using the pin-stirrer, the applied shear (1500 rpm rotating shaft

254   speed) and cooling rate (30 °C/min; 20 °C at water bath) were kept constant throughout. This

255   high cooling rate that the particles were exposed to within the pin-stirrer makes it very difficult

256   to make a direct comparison of the fluid gel samples formulated using the rheometer production

257   method, due to the cooling constraints of the rheometer equipment (maximum cooling rate = 6

258   °C/min). Thus, comparisons of the respective fluid gel samples were based solely on their shear

259   rates during production.

260   The post-production flow behaviour was measured, by recording the viscosity of the fluid

261   gel sample (2 wt. %) 24 hours after production. The data is shown on a log-log scale in Fig. 2,

262   where an almost identical shear-thinning behaviour is observed to that of the fluid gel sample

263   produced using the rheometer at the calculated equivalent shear rate (50 s^{-1}). The conclusion

264   therefore is that the particle sizes are similar independent of the method of production, so long

265   as the shear rate during gelation is comparable.

266

267   3.1.2.   Material response over time

268   Fluid gel systems involve inter-particle interactions that persist post-processing, and further

269   develop or ripen for a significant time after processing completion. To understand the time

270   scale over which this strengthening process occurs kinetic studies were implemented. For both

271   the 2 wt. % *LA* gellan gum fluid gels produced using the rheometer and the 2 wt. % *LA* gellan

272   gum fluid gels produced using the pin-stirrer, an oscillatory frequency of 1 Hz and a 1 % strain

273   were applied to the fluid gel samples after production following a 1 hour resting period, and the

274   elastic and viscous modulus responses were measured as a function of time. The results are

275   shown in Fig. 3.

11

276   Fig.3 shows that for the fluid gel sample produced using the rheometer the storage modulus

277   remains at a constant level over time. The viscous modulus also remained constant over time,

278   except for a 15 Pa decrease at the start. This could be due to the low shear rate allowing more

279   inter-particle interactions to occur during formation, but it is likely to be a mere measurement

280   error during the time period where the oscillation experiment is first being established.

281   Particularly, since a similar decrease was not observed with the viscous modulus recorded for

282   the fluid gel sample produced using the pin-stirrer (Fig. 3).

283   In contrast, the data shown in Fig. 3 for the fluid gel sample produced using the pin-stirrer

284   exhibits much higher viscous and elastic moduli, although both remain constant over time. This

285   is accounted for by the high cooling rate (30 °C/min) used with the pin-stirrer method where the

286   gelation process dominates, resulting in the production of fluid gels that have high storage

287   modulus characteristics, and thus more solid-like behaviour.

288   The results indicate that after a 1 h resting period, both fluid gel sample networks have

289   reached their maximum inter-particle interaction levels, with no further strengthening or

290   ripening occurring thereafter.

291   Overall, each of the fluid gel samples produced by the two methods display weak gel

292   behaviour. This can be explained structurally; closely packed particle interactions allow an

293   elastic network to form at rest, whilst under shear, they are disrupted allowing material flow.

294

295   3.1.3.   The coil-helix transition

296   Low-amplitude oscillation measurements were performed on the fluid gel samples produced

297   using each production method after their formation, during heating and cooling. These enabled

298   the coil-helix transition temperatures to be identified, allowing an improved understanding into

299   the molecular ordering.

12

300   Figure 4 shows the elastic and viscous moduli at 1.585 Hz for 2 wt. % low acyl gellan gum

301   fluid gels, produced within a rheometer (50 s^{-1} applied shear rate, 3 °C/min cooling rate)

302   following frequency tables from 0.1 – 10 Hz performed every 10 °C during temperature heating

303   (a) and cooling (b) steps between 20 – 90 °C. On heating, the aggregated regions must be

304   disassembled before the helix-coil transition can occur. This is represented by the steep

305   decrease in the elastic modulus between 20 – 30 °C, with the helix-coil transition occurring

306   thereafter between 30 – 70 °C. Above 70 °C, the solution exists in the disordered coil state.

307   However, a steep increase in the elastic modulus is evident between 80 – 90 °C. The elastic

308   modulus data point at 90 °C is most likely to be a result of sample evaporation, which is possible

309   when performing tests of this type at high temperatures that are close to the boiling point of

310   water, even with the appropriate evaporation prevention methods in place. This leads to an

311   increased concentration of the gellan aqueous solution, forcing the disordered coils closer

312   together causing them to have restricted mobility, and hence increased solid-like character.

313   The midpoint temperature (T_m) is the midpoint between the two linear regions

314   corresponding to disordered and ordered polymer conformations. For the helix-coil transition in

315   Fig. 4a, T_m = ~ 50 °C. T_m represents the temperature at which the number of moles of saccharide

316   residues in the disordered and ordered states are equal, and where the events of helix growth

317   (zipping-up) and decay (un-zipping) are occurring at equal rates (Goodall & Norton, 1987).

318   During the frequency test on cooling (Fig. 4b), we essentially monitored the formation of a

319   quiescently cooled gel, since the test was performed immediately after melting the fluid gel, and

320   with no external shear force applied, from 90 – 20 °. As with heating, three distinct

321   conformational regions exist in the elastic modulus data: 90 – 60 °C, the solution exists in the

322   disordered state; 60 – 40 °C, the coil-helix transition occurs; 40 – 20 °C, formation of an ordered

323   structure via aggregation. For the coil-helix transition, the T_m = ~ 50 °C. The coil-helix transition

324   has been described as a dynamic equilibrium where the rate constant at T_m is zero, and on

13

325   decreasing temperature the likelihood of growth events is increased relative to those of decay

326   (Norton et al., 1983). Thus molecular rearrangements to both quiescent and fluid gels, via decay

327   and growth mechanisms are reduced on decreasing temperature below T_m.   The growth

328   mechanism that is most likely to be responsible for the formation of the quiescent *LA* gellan gel

329   in Fig. 4b, involves intermolecular association of the polymer molecules in the solution during

330   the coil-helix transition, which result in the formation of small, soluble clusters of chains. As the

331   extent of association increases (as the temperature is reduced) these clusters grow, until

332   ultimately they interlink to span the entire volume of the solution and form a continuous

333   crosslinked network (Morris et al., 2012).

334   For both Figs. 4a and b, the elastic modulus values across the temperature range are greater

335   than those recorded for the viscous modulus from the point of gel ordering completion to the

336   region where the solution is first in the disordered coil state. During this latter region, the values

337   follow identical pathways. Interestingly, the elastic modulus values reported for the formation

338   of the quiescent gel are of a higher magnitude ($\sim$ 5 times), than those for the melting of the fluid

339   gel. In the absence of shear, the re-ordering during cooling can take place between particles (as

340   well as within particles) i.e. interparticulate helices form, thus the products require a greater

341   force to allow the particles to move past one another and store more energy (greater elastic

342   modulus) (Garrec & Norton, 2012).

343   It has been suggested that the *LA* gellan gum fluid gel particles consist of an internal

344   polymeric network, and therefore the elasticity of individual particles is equivalent to that of the

345   quiescently cooled gel (Caggioni et al., 2007). The fact that very similar viscoelastic responses

346   with temperature were observed for the fluid gel produced under shear using the rheometer,

347   and with the quiescent gel formed on cooling in the absence of shear (Figs. 4a and b) suggests

348   that this is correct.

349   The fluid gel sample produced using the jacketed pin-stirrer exhibited similar rheology with

350   temperature on heating and cooling to that formed using the rheometer. On heating and

351   cooling, the three distinct conformational regions were also observed in the elastic modulus

352   data, and as expected the temperature ranges for each were identical for both experiments.

353   These were: 20 – 30 °C, dissembling of the aggregated stable structures (heating) and formation

354   of an ordered structure (cooling); 30 – 50 °C, helix-coil transition (heating) and coil-helix

355   transition (cooling); 50 – 90 °C, the solution exists in the disordered coil state. For each system

356   (fluid and quiescent respectively) the mid-point temperature was T_m = ~ 40 °C. The 10 °C

357   difference in this mid-point temperature value compared with that observed for the gels

358   produced using the rheometer method, is accounted for by the thermal lag in the oscillatory

359   measurements due to the mass of the geometry used. However, overall these values together

360   with the helix-coil and coil-helix transition ranges recorded for each fluid gel production method

361   agree well with the gel phase transition temperatures reported in the literature for *LA* gellan

362   gum (García et al., 2011). They also reinforce the ordering initiation temperature of ~ 42 °C

363   from the viscosity measurements during *LA* gellan gum fluid gel formation within the rheometer

364   (Figure 1).

365   The fluid gels formed using the jacketed pin-stirrer also had elastic modulus values across

366   the temperature range that were greater than those recorded for viscous modulus. As

367   described for the former production method, the elastic modulus values reported for the

368   quiescent gel using this method were also of a higher magnitude than those for the melting of

369   the fluid gel. Additionally, the viscoelastic measurements obtained using this method were

370   found to be approximately 3 times larger than those obtained when using the rheometer to

371   produce a fluid gel. This can be explained through the fact that the fluid gel sample formed

372   using the jacketed pin-stirrer was allowed to rest for 24 hours after production, prior to

373   rheological testing where this is not possible when the fluid gel is made directly on the

374   rheometer itself.

15

375   For each production method, the fluid gel samples displayed similar viscous modulus

376   measurements. The fluid gels formulated using the jacketed pin-stirrer displayed on melting

377   viscous modulus values almost identical to those of the elastic moduli up until the end of the

378   helix-coil transition at 50 °C. Whilst, the fluid gels formulated using the rheometer displayed on

379   cooling viscous modulus values almost identical to those of the elastic moduli up until the start

380   of the coil-helix transition at 60 °C. Thereafter, a steady increase was observed, which is

381   reflective of the samples' elastic distortion of the disentangled/entangled network during

382   structural rearrangement, in response to the low-amplitude oscillatory deformation

383   perturbation (Morris et al., 2012).

384

385   3.2. Texture analysis and post-production exposure to acidic environment

386   The previous section compared the two methods of fluid gel production via the use of a

387   rheometer and a jacketed pin-stirrer. The rheometer is advantageous in that it provides a

388   constant and near uniform shear field, with a high degree of control. However, the technique is

389   not particularly typical of industrial processes. The jacketed pin-stirrer method on the other

390   hand, is more representative for commercial processes, thus this was the method chosen for the

391   direct and post-production acid exposure experiments.

392   Post-production exposure of the *LA* gellan gum fluid gels to an acidic environment can be

393   used to assess their response to prolonged acid conditions that are similar to those found in the

394   stomach during digestion. It is widely accepted (Yamamoto & Cunha, 2007; Norton et al., 2011)

395   that *LA* gellan is acid-sensitive. Research (Norton et al., 2011; Bradbeer et al., 2014) has shown

396   that *LA* gellan gum gel strength increases on exposure to an HCl acid soak. This is a result of the

397   existing gel networks already partially formed within the gels prior to soaking being reinforced

398   on acid exposure. Ordering and aggregation between individual hydrocolloid chains occurs

399   immediately upon acidification (Moritaka et al., 1995). An increase in gel strength was observed

16

400   qualitively with the 1 wt. % *LA* gellan gum fluid gels (1500 rpm shaft speed; 100 ml/min pump

401   rate; 20 °C water bath) post HCl acid bath soak, by the change in gel state from a fluid to a solid.

402   Fig. 5 shows the resulting true stress/true strain curves following compression tests performed

403   on these solid gels, as a function of the number of runs that the gel was processed through the

404   pin-stirrer unit during fluid gel formation. Each of the samples displayed purely brittle fracture

405   behaviour, with a rapid decrease in the applied stress once the gels fail at strains between 40 –

406   42.5 %, where a clear fracture point is observed.

407   In terms of gel stiffness and their resistance to fracture after soaking in acid, minimal

408   differences (0.0260 MPa and 0.0250 J/m^2 respectively) were found between the fluid gel

409   samples collected after the first two cycles through the pin-stirrer. However, for the sample

410   collected after three runs, a reduction in gel strength and stiffness (0.5907 MPa) was observed; a

411   smaller force (0.2347 J/m^2) was needed to induce fracture (Fig. 5). This sample was exposed to

412   the shear force and cooling environment within the pin stirrer for a greater time period than the

413   other samples. It has been reported that interparticle interactions diminish with continued

414   cooling and shearing during processing, although some interactions will still persist (Cox et al.,

415   2009). Therefore, it would be expected for the fluid gel structure to be weaker and less

416   responsive to the acid exposure, resulting in a weaker acid gel. In addition, the cooling rate

417   implemented (32 ml/min) during the fluid gel production was fairly high. Generally, during high

418   cooling rates the gelation process dominates and fluid gels are produced that have high storage

419   modulus characteristics, and thus more solid-like behaviour indicating larger and more

420   irregularly shaped particles (Cox et al., 2009), essentially resulting in a chopped quiescent gel

421   rather than a fluid gel. As a result, a heterogeneous fluid gel sample is produced, consisting of

422   fluid gel particles along with broken-up fragments, which ultimately leads to a weaker gel with a

423   reduced packing fraction. This together with the diminished interparticle interactions within the

424   gel structures reduces the subsequent response to the acid exposure, explaining why the weak

425   gel behaviour is observed.

17

426

427   3.3. Direct exposure to acidic environment

428   A variety of acid fluid gel structures can be generated through the direct addition (dropwise)

429   of 0.5 % HCl acid to the aqueous *LA* gellan solution at natural pH, during the production process

430   prior to shearing inducing a range of pH environments. However, the rate of aggregation using

431   this direct HCl addition method is expected to be much higher than the rate achieved by

432   thermally, or ionically induced gelation. This suggests that the extent of cross-linking between

433   the polymer chains (and therefore the elasticity and strength of the overall *LA* gellan acid fluid

434   gel structure) during direct addition of HCl becomes less efficient than when cross-linking occurs

435   in thermally or ionically set gels.

436   Figure 6 shows flow curve data as a function of pH, for 1 wt. % *LA* gellan gum fluid gels

437   produced within the jacketed pin-stirrer (1500 rpm shaft speed, 100 ml/min pump rate, 15 °C

438   water bath), measured 24 hours after production. In terms of viscosity response on pH

439   reduction the acid gels with acidity from natural pH – pH 3 displayed much higher resistances to

440   flow than those at pH 2. Initial acidification from neutral pH to pH 3.5 causes a large increase in

441   ordering and aggregation to occur between the individual hydrocolloid chains immediately upon

442   acidification (Moritaka et al., 1995), resulting in marked increases in viscosity. However, on

443   further decrease in pH below the pK$_a$ of the glucuronate residues of gellan, at ~ pH 3.4 (Haug,

444   1964), over-structuring occurs, causing the gels at pH 2 to be extremely weak and turbid, and

445   showing precipitation of the polymer (Moritaka et al., 1995). As a result an almost sponge-like,

446   weak structure is created rather than a stronger homogeneous one. It is very likely that gel

447   structuring at pH 2 is disrupted by the shear applied during the acidification process, and that

448   much lower levels of shear-induced disruption would occur for the mixing conditions found in

449   the stomach. Singh (2007) reported that the shear force on the surface of a food particle (~

450   0.00043 N) is insignificant in comparison to the crushing or grinding force due to the walls of the

451   stomach (0.2 N).

452   At pH 2, the 1 wt. % *LA* gellan gum acid fluid gel displayed non-Newtonian shear-thinning

453   flow behaviour i.e. the fluids decrease in viscosity with increasing shear stress (Fig. 6). However,

454   for the respective gels with acidities from natural pH – pH3, a range of individual viscosity yield

455   stresses appear to exist between the approximate shear stress range of 0.07 – 1 Pa. For each

456   pH, a minimum force exertion (shear stress) was required to induce flow behaviour. As soon as

457   the yield stresses had been exceeded, the liquid samples then proceeded to display shear-

458   thinning behaviour (Cui, 2004). This pseudoplastic flow behaviour is characteristic of the stiff

459   polysaccharide chains present in gels of this type under such reduced pH conditions (Cui, 2004).

460

461   Phase separation is observed as a decrease in the elastic moduli values at 1.00 Hz in Fig. 7

462   for the 1 wt. % *LA* gellan gum fluid gels produced within the jacketed pin-stirrer (1500 rpm shaft

463   speed, 100 ml/min pump rate, 15 °C water bath) at pH 2, confirmed by the observation of

464   turbidity at the pH level where their strength began to decrease.

465   The elastic modulus data, which was measured during a temperature ramp (10 – 90 °C),

466   shows (Fig. 7) that, for the remaining pH values, the elasticity and strength of the gels increases

467   systematically with pH reduction. This demonstrates that the gels become stronger, as a result

468   of greater numbers of cross-links between the polymer chains promoted at the lower pH

469   conditions, and is in good agreement with the flow curve data reported in Fig. 6. Albeit in Fig. 6,

470   the pH 4 acid fluid gel displays the greatest strength amongst the gels, compared to the pH 3

471   acid fluid gels in Fig. 7.

472   The elastic modulus data (Fig. 7) shows that with decreasing pH gelation occurs sooner. This

473   is observed as the onset of gelation at higher temperatures for the acid fluid gels at lower pH.

474   The elastic moduli recorded for the acid fluid gel samples at acidities of natural pH and pH4 are

475 similar with increasing temperature. Disassembling of the aggregated stable structures occurs,

476 breaking inter-helical bonds etc. (pH 5: 10 – 40 °C; pH 4: 10 – 50 °C), before moving into the

477 conformational helix-coil transition phase (pH 5: 40 – 70 °C; pH 4: 50 – 80 °C), and finally the

478 region where the solution exists in the disordered coil state (pH 5: 70 – 90 °C; pH 4: 80 – 90 °C).

479 The elastic moduli recorded at pH 3 exhibit extended fluid gel deformation events, as a

480 consequence of the speed of the gelation process. Between 10 – 40 °C, a constant elasticity

481 level is observed, which represents a minimum particle size unaffected by cooling or heating

482 below the final gelation set point at 40 °C. During heating between 40 and 70 °C de-fixing of

483 the gelled particle structures proceeds, disordering the hydrocolloid chains within the particles,

484 creating disordered chain segments at the surface of the particle giving them a 'hairy' nature

485 (Cox et al., 2009). The completion of the melting occurs after 90 °C. Between 70 and 90 °C, full

486 conformational disordering and aggregate break up takes place. The absence of a region,

487 where the solution exists in the disordered coil state is explained by the fact that during

488 formation on heating under such low pH conditions, ordering and aggregation between the

489 individual gellan polymer chains occurs immediately upon acidification (Moritaka et al., 1995).

490 The effects of pH on gellan gelation have been associated with the lower charge density of

491 chains at lower pH values (Horinaka et al., 2004). They suggested that, since the carboxyl group

492 included in the gellan chain is a weak acid group, and that the degree of dissociation of carboxyl

493 groups in aqueous systems is dominated by the dissociation constant, the lower the pH value,

494 the smaller the fraction of dissociated carboxyl groups, making the gellan a less anionic

495 polyelectrolyte. It is then expected that the less anionic chains aggregate with one another

496 more easily, due to the lower electrostatic repulsion. In addition, the decrease in electrostatic

497 repulsion between the intramolecular segments may result in the suppression of gellan chain

498 expansion (Yamamoto & Cunha, 2007), making association even easier. This overall ease of

499 aggregation caused by decreasing the pH via the direct addition of HCl acid explains the

500 decrease in time to reach the gel point, the more densely linked structure at equilibrium and

501 consequent changes in gel strength, deformability and turbidity.

502

503   The *LA* gellan gum acidified fluid gels were assessed post-production in terms of their

504 response to a prolonged exposure to an HCl acid environment as described in Section 3.2.

505 Quiescent *LA* gellan gum gels were also exposed in this way for fluid gel comparison. Fig. 8

506 shows the resulting true stress/true strain curves following compression tests performed on the

507 gel samples, as a function of pH. Each of the samples displayed purely brittle fracture

508 behaviour, with a rapid decrease in the applied stress once the gels fail at strains between 20 -

509 35 %, where a clear fracture point is observed.

510   The gelation of gellan can be induced by a reduction in pH, with Grasdalen and Smidsrød

511 (1987) describing HCl acid as "the most potent gel-former". However, the variation in gel

512 strength with increasing concentration of acid is not monotonic. The addition of acid results in

513 a large increase in break stress (Picone & Cunha, 2011) down to pH 3.5, where it then proceeds

514 to decrease with further pH decline (Norton et al., 2011), until the gels become extremely weak

515 and exhibit precipitation of the polymer by pH 2 (Moritaka et al., 1995). This behaviour is

516 particularly evident in Fig. 8, where the *LA* gellan acid fluid gels at acidities of natural pH were

517 stiffer and more resistant to fracture than those at lower pH levels. In fact, for the samples with

518 acidities of pH 3 and pH 2, the compression tests were unable to be performed, since the gels

519 were too weak to be tested, following their collapse on removal from the visking tubing.

520   For both the 1 wt. % *LA* gellan gum quiescent and acid fluid gels at acidities of pH natural

521 and pH 4, we observe a reduction in the failure stress and a shift in strain to lower values with

522 decreasing pH. The shift in strain values is generally indicative of an increase in gel brittleness,

523 whilst the reduction in break stress can be attributed to the combination of the acid

524 concentration and low gellan concentration within the sample, giving rise to a weak network.

525   Minimal differences (0.0565 MPa and 0.0092 J/m^2 respectively) are observed between the

526   total work of failure and bulk modulus data for the *LA* gellan gum quiescent and acid fluid gels

527   at pH 4. However, slightly larger differences (0.0615 MPa and 0.2317 J/m^2 respectively) were

528   observed for the samples at their natural pH, with the quiescent gel sample having both the

529   larger break stress and strain. This observation further reinforces the outcomes of the elasticity

530   data collected for that of the 2 wt. % *LA* gellan gum quiescent and acid fluid gels at their natural

531   pH, in which higher values were reported for the quiescent gel. This can be explained by the

532   theory that in the absence of shear (i.e. for quiescent gels), the re-ordering during cooling can

533   take place between particles (as well as within particles) i.e. interparticulate helices form, thus

534   the products require a greater force to allow the particles to move past one another and store

535   more energy (greater elastic modulus) (Garrec & Norton, 2012). In turn, stronger gels are

536   formed that exhibit greater resistance to deformation past the elastic limit.

537

538   4.   Conclusions

539   With the potential for use of fluid gels in low fat and reduced calorie foods, as well as within

540   self-structuring satiety based food formulations that take advantage of the natural digestive

541   processes, their formation and properties were explored.

542   We have shown that by controlling the material (*LA* gellan gum and HCl acid

543   concentrations) and process (shear and cooling rates during fluid gel production) parameters it

544   is possible to manipulate the properties and size of individual particles as well as the

545   interactions/bridging between them. Both the fluid and acidified fluid gels produced using

546   these production methods displayed non-Newtonian shear-thinning flow behaviour. Very close

547   similarities were observed between the fluid gels produced by both methods, suggesting that

548   the two distinctive processes are capable of producing particle sizes on similar scales.

549   It was shown through the rheological and texture analysis data that compared with the fluid

550   gels, the quiescent gels were characteristically stronger, due to the formation of interparticulate

551   helices in the absence of shear on cooling. Fluid gels are said to have fewer and shorter helices

552   than quiescently cooled gels due to the disruption of molecular ordering caused by the applied

553   shear during their production (Garrec & Norton, 2012). Despite this, the coil-helix transition and

554   mid-point temperature data recorded for both gel types, fell within the same range (30 – 70 °C),

555   which was agreeable with the literature (García et al., 2011).

556   Post-production and direct exposure of the fluid gels to an acidic environment resulted in an

557   increase in gel strength. However, the variation in gel strength with increasing concentration of

558   acid (via direct-addition) was not monotonic, with phase separation of the polymer and solvent

559   proceeding for the fluid gel samples with acidities below pH 3. The elastic modulus data for the

560   acidified fluid gels showed that decreasing the pH, leads to a subsequent reduction in the time

561   taken for gelation to occur.

562   These findings are promising as they clearly demonstrate that the structuring of *LA* gellan

563   gum fluid gels can be controlled by both the process used for their production and by exposure

564   to an acidic environment. The fluid gel remains liquid and therefore has potential for inclusion

565   in the structure of many different types of foods, and yet still structures when exposed to acidic

566   conditions mimicking the stomach environment in the same way as the solid quiescent gel,

567   potentially providing an increase in satiety level.

568

572

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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.
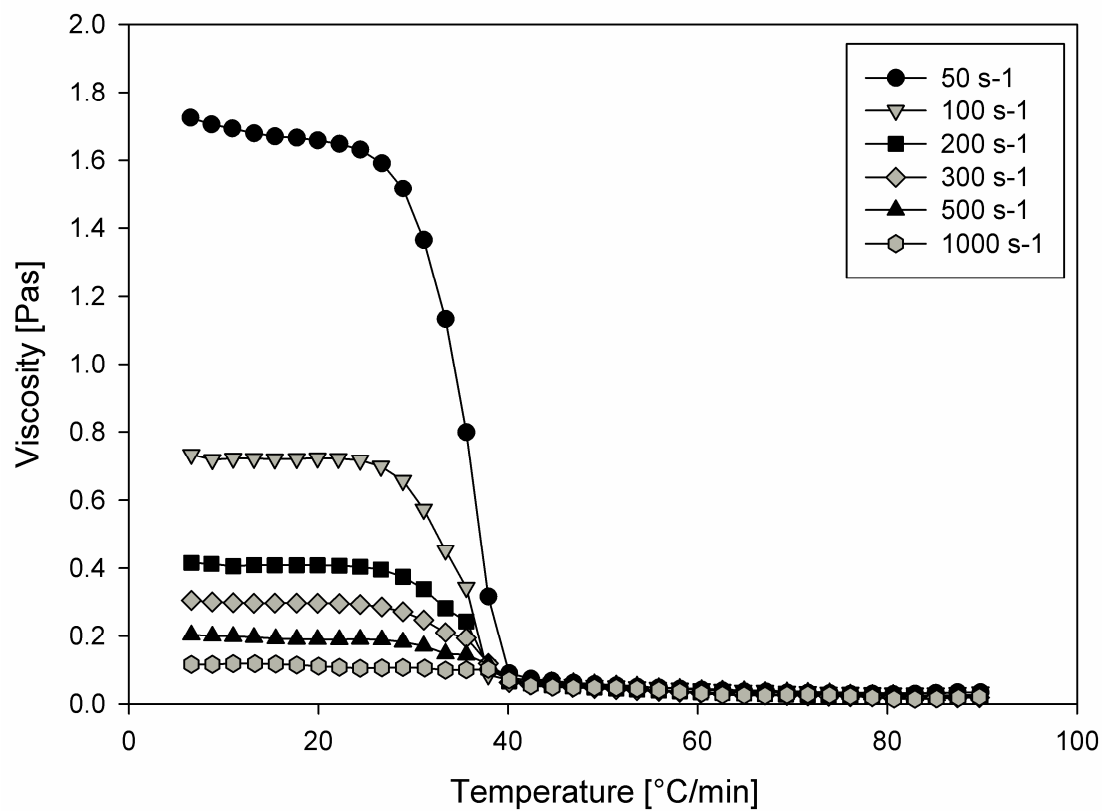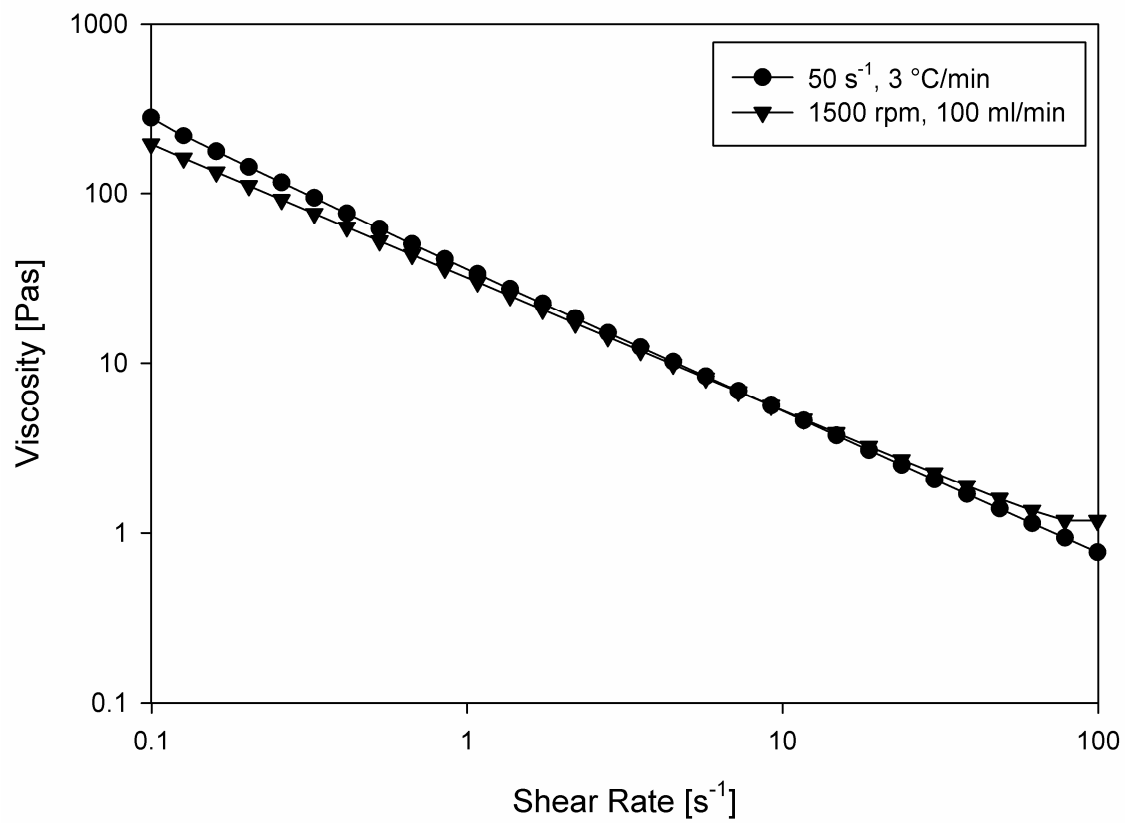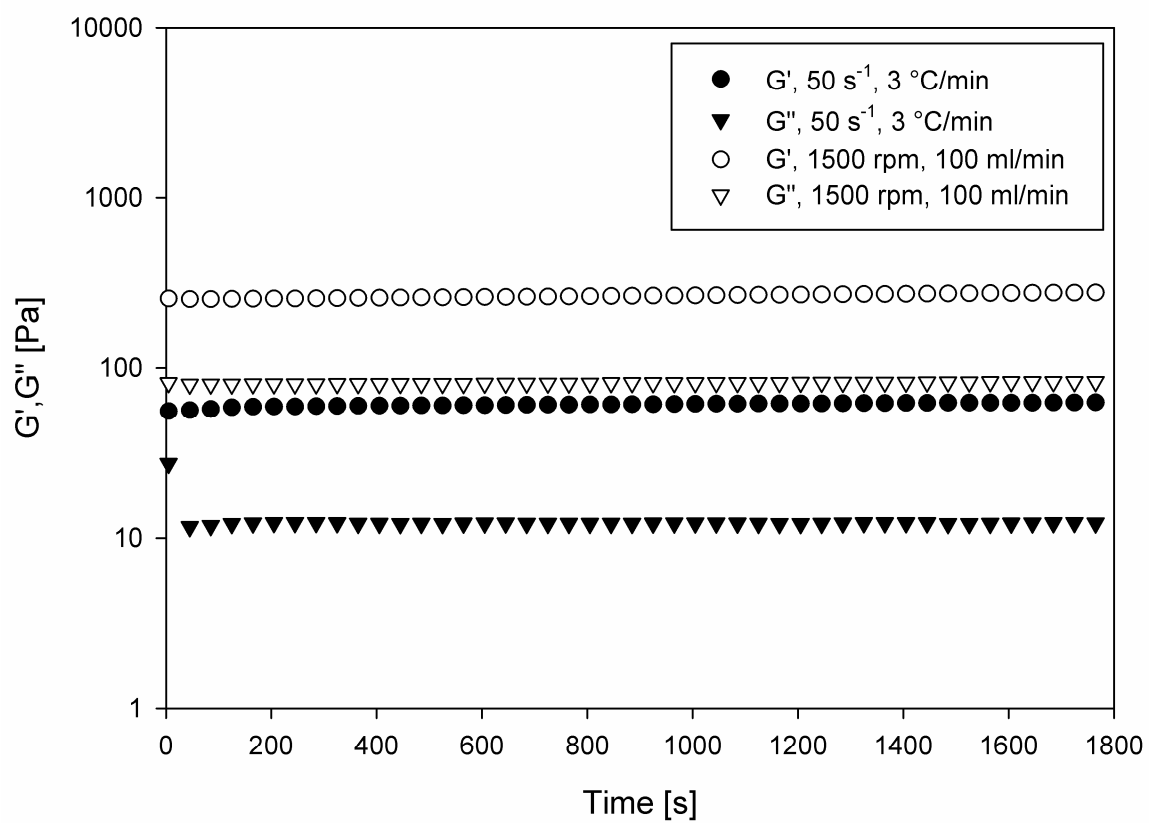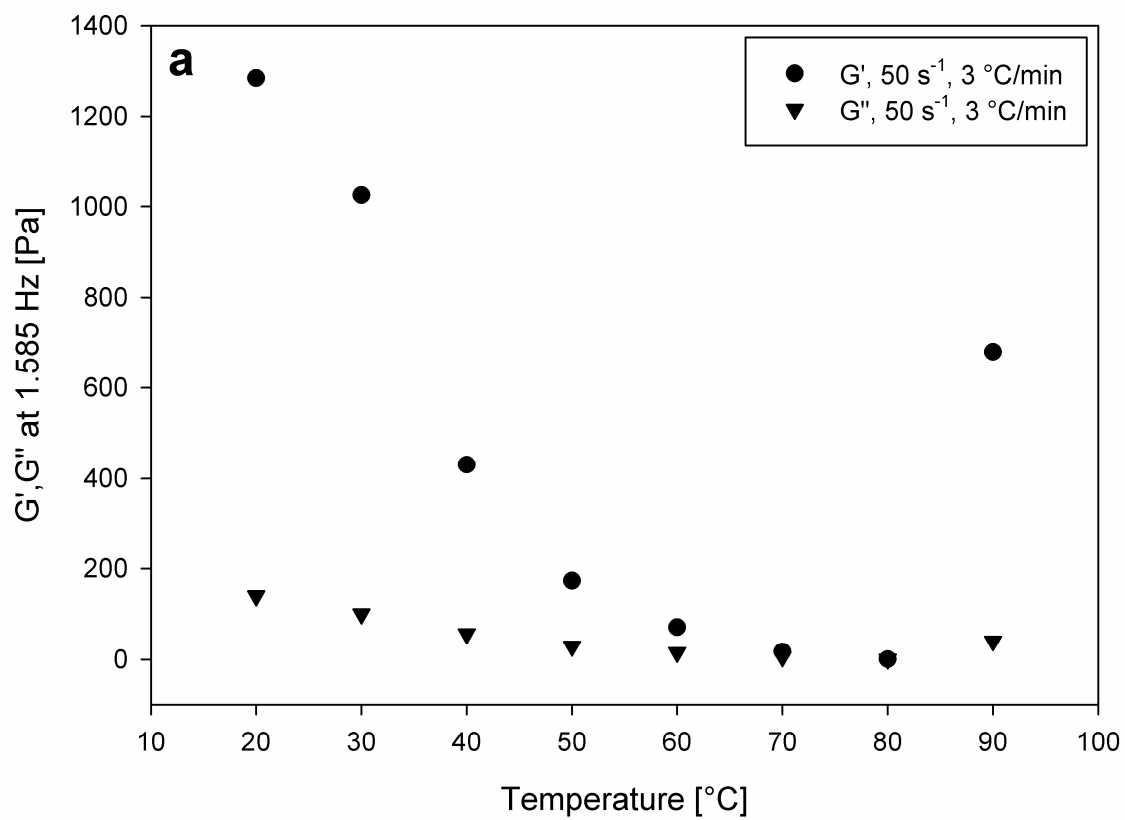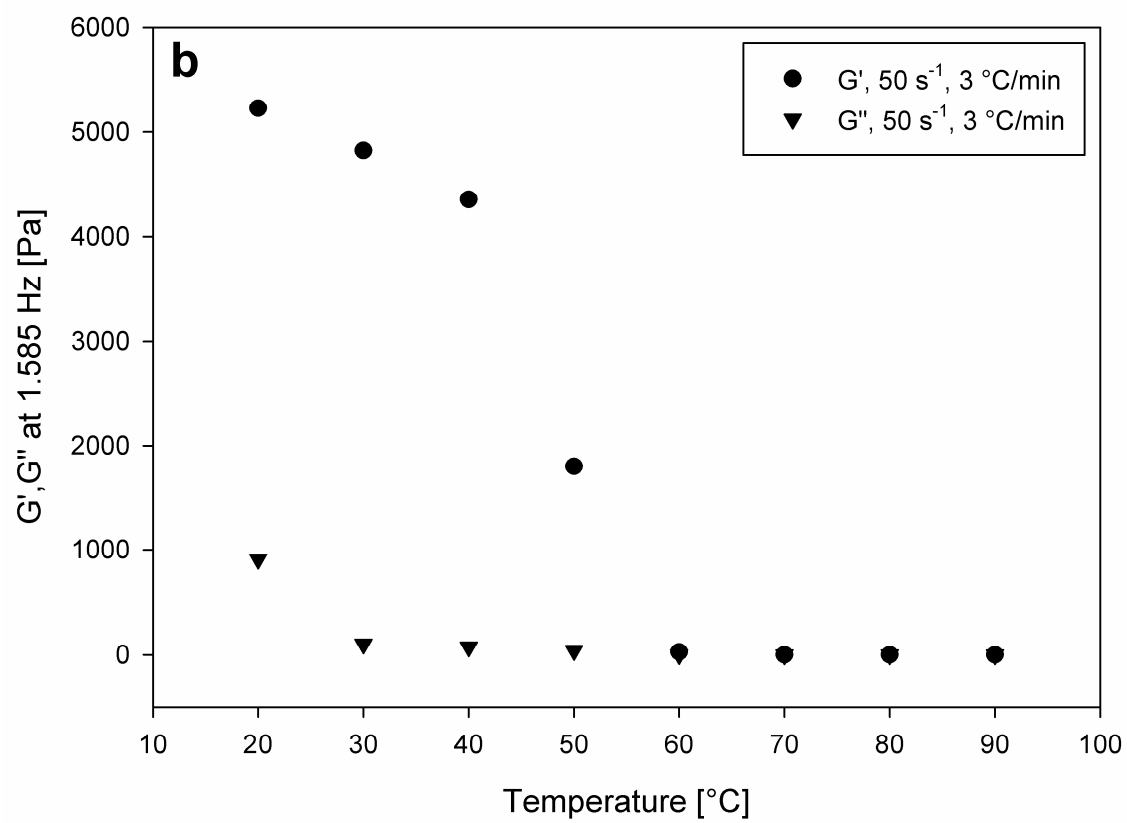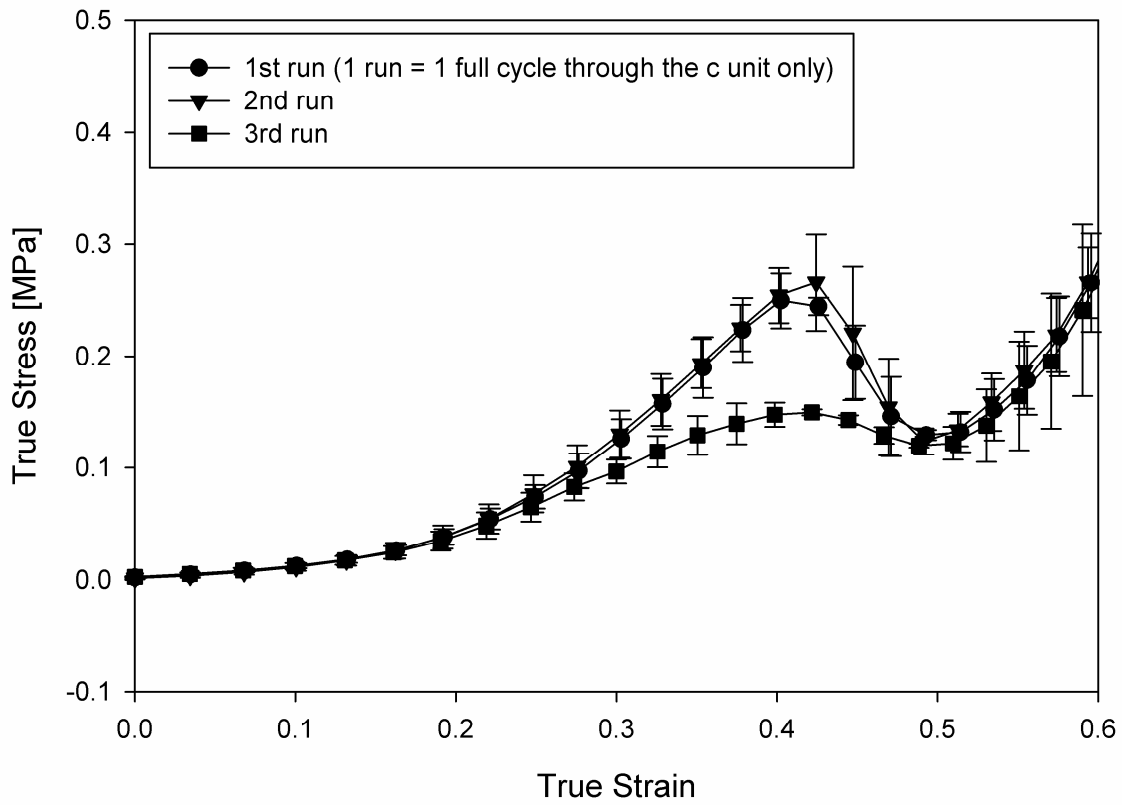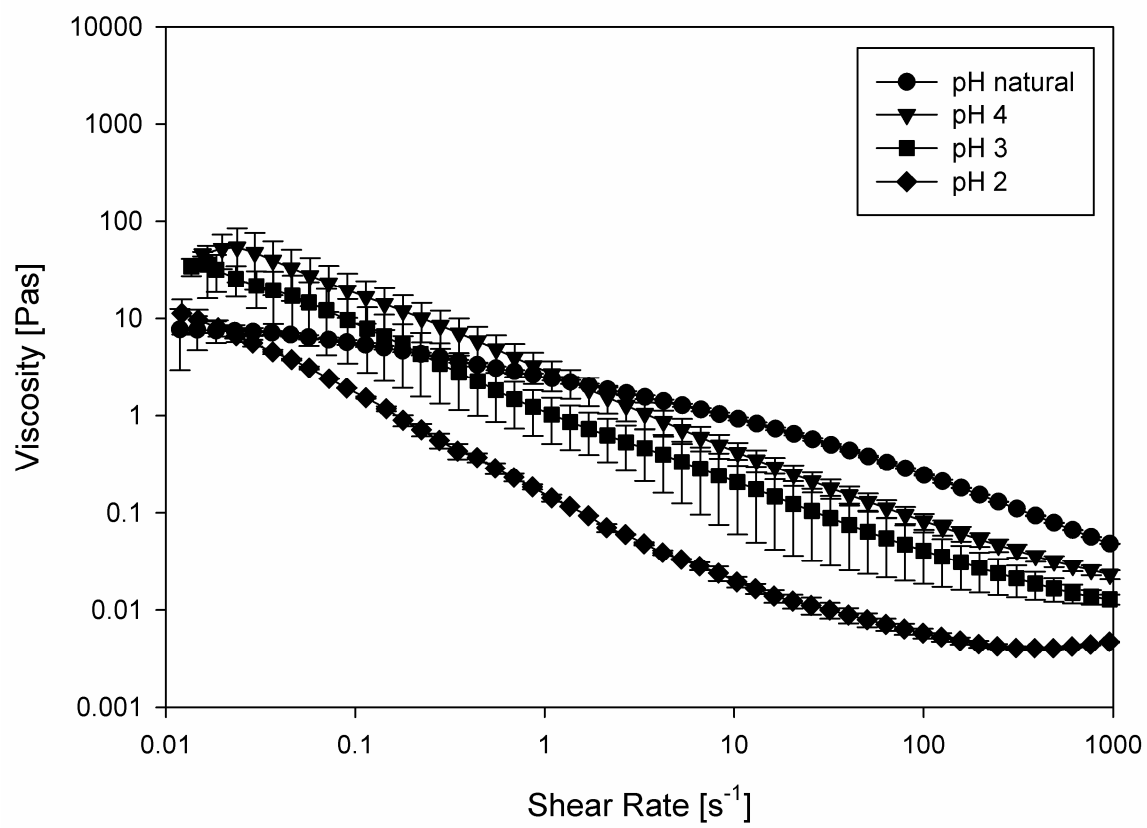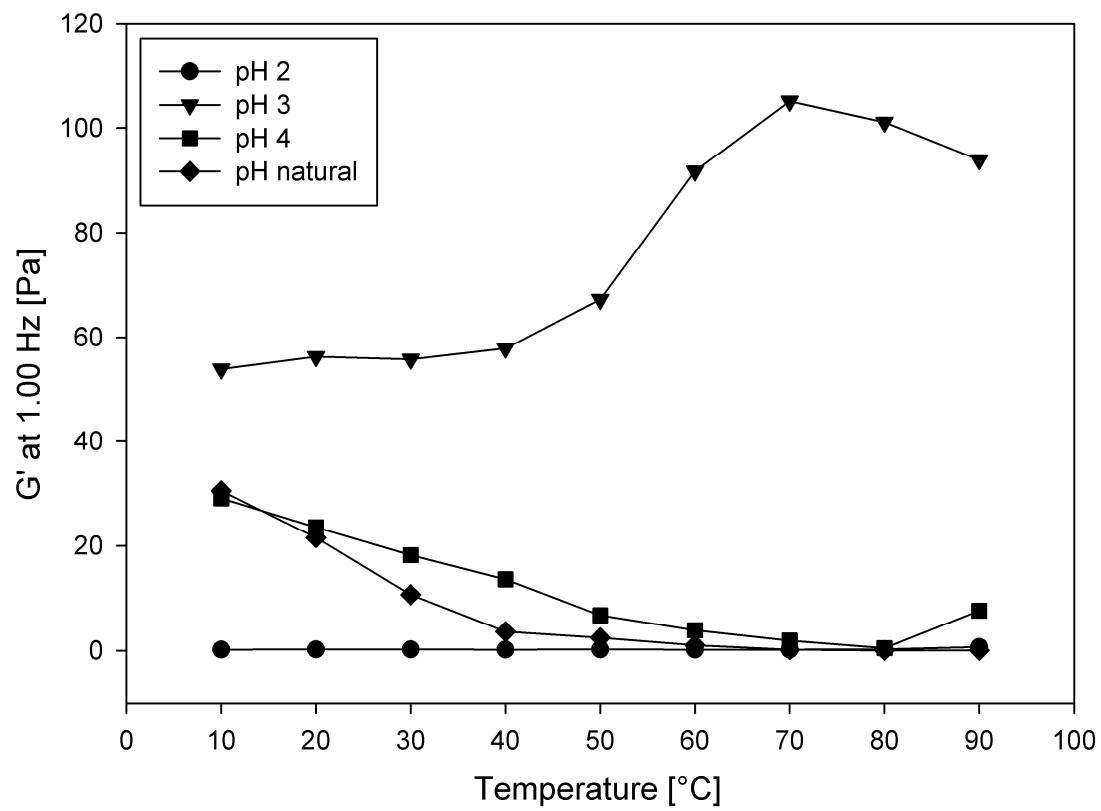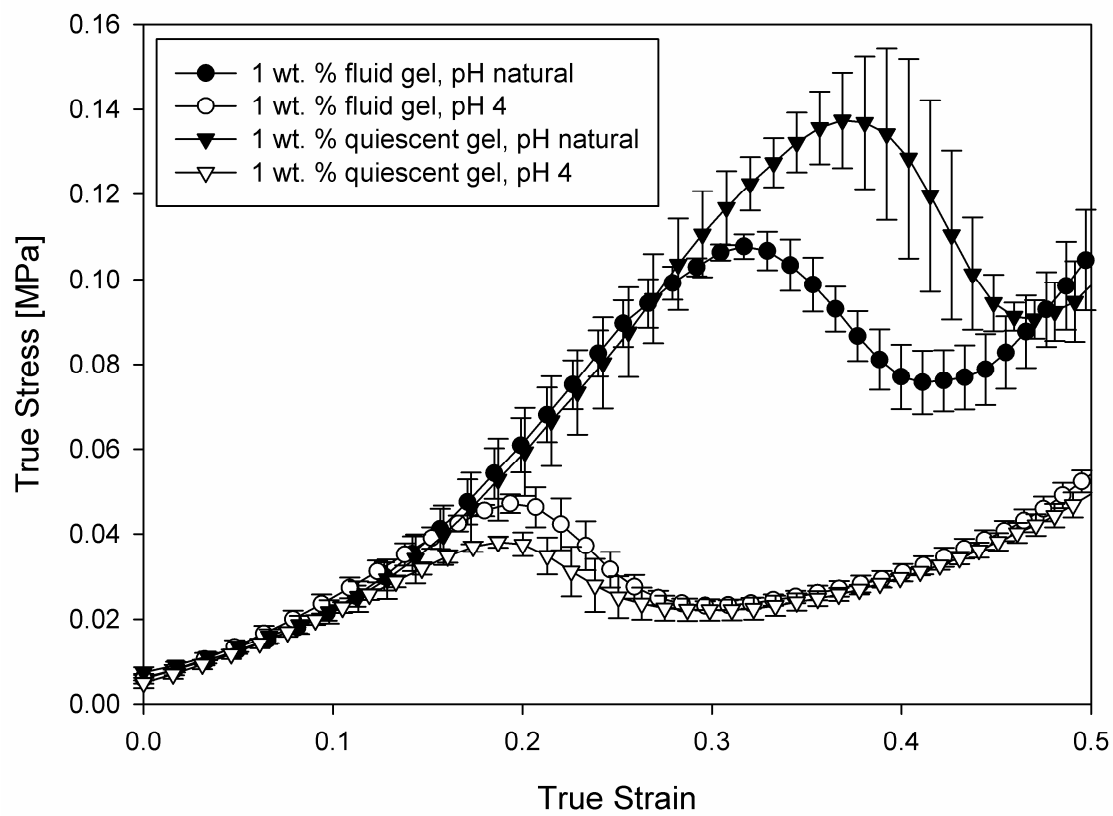
Highlights

- 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.