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Acid gelation of gellan: Effect of final pH and heat treatment conditions

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Abstract

The influence of polysaccharide concentration, final pH (2.0–4.0) and heat treatment temperature (70 or 90 °C/30 min) on the rheological properties, water-holding capacity (WHC), turbidity and microstructure of gellan gels formed by acidification with glucono-δ-lactone (GDL) was evaluated. In addition the gelation kinetics was monitored by oscillatory rheological measurements. Heating the solutions, raising the polymer concentration and lowering the final pH decreased the time taken to reach gel point. Failure stress increased at lower pH values, higher polysaccharide concentration and with the application of heating before acidification. However, gels heated after acidification showed decreased failure stress. All the gels were transparent and the WHC improved at higher concentrations and pH values or at lower pH values and gellan concentrations. The microstructure and turbidity showed good correlation with the mechanical properties, which could explain the influence of the process conditions on the gel properties.

Keywords: Gellan gum; Gelation; Rheology; Acidification; Microstructure

1. Introduction

Polysaccharides are widely used in the replacement of ingredients, particularly in low fat and reduced calorie formulated foods (Tang, Lelievre, Tung, & Zeng, 1994). Bacteria have become an increasingly important source of polysaccharides, although most of them are not of commercial value since they do not offer significant advantages over existing alternatives. Gellan gum is an exception amongst the bacterial polysaccharides due to its ability to modify rheological properties at very low levels of use, providing a wide range of textures, depending on the concentration and medium conditions. In the food industry, an ingredient that allows the design of new and differentiated food products is highly desirable (Sanderson, 1990). However, the successful application of gellan polymer as a gelling agent to provide desired textural properties in foods depends on a thorough understanding of the relationships between the mechanical properties and interactions amongst gel-forming constituents at the molecular level (Tang, Tung, & Zeng, 1995).

Gellan gum is an extracellular polysaccharide produced by the bacterium *Sphingomonas elodea*. It is composed of repeating tetrasaccharide (1,3-β-D-glucose, 1,4-β-D-glucuronic acid, 1,4-β-D-glucose, 1,4-α-L-rhamnose) units containing one carboxyl side group. In aqueous solution at high temperatures gellan polymers are in a disordered single-coiled state. Cooling of the gellan sol promotes the formation of a threefold left-handed double helix, stabilized by internal hydrogen bonding (Chandrasekaran & Radha, 1995). Coil-helix conformational transition occurs in a temperature range from 30 to 50 °C, depending on the ionic strength of the solution (Chandrasekaran & Radha, 1995; Horinaka, Kani, Hori, & Maeda, 2004; Miyoshi, Takaya, & Nishinari, 1995; Nickerson, Paulson, & Speers, 2003; Rodríguez-Hernández, Durand, Garnier, Tecante, & Doublier, 2003). After this transition, the gellan double helixes can be associated in the presence of cations to form junction zones, which can aggregate and lead to the

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formation of an interconnected three-dimensional gel network. During this step the sol is converted into a solid state (gel). The gelation process is dependent on the type of cation, ionic strength, temperature and polymer concentration (Sanderson, 1990), and the network structure of the gellan gels can also be modified by heating the solutions at temperatures higher than the gel–sol transition point (Yoshida & Takahashi, 1993).

It is well known that the gelling ability of traditional gelling agents such as agarose and carrageenan is diminished at low pH. Research and development into ingredients that could form gels at low pH values is important for the production of products such as dessert jellies containing fruit juices (Moritaka, Nishinari, Taki, & Fukuba, 1995). It is noteworthy that aqueous systems of gellan maintain the gel state over a wide pH range in comparison with those made from other polysaccharides. Since gellan is an anionic polyelectrolyte, the pH may affect the conformation of the gellan chains in aqueous systems in two ways. One is the shielding effect caused by the electrostatic repulsion between the carboxyl groups found in the gellan units, as reported for other cation species (Chandrasekaran & Radha, 1995; Gunning, Kirby, Ridout, Brownsey, & Morris, 1996; Ikeda, Nitta, Temsiripong, Pongsawatmanit, & Nishinari, 2004; Miyoshi, Takaya, & Nishinari, 1996; Miyoshi, Takaya, & Nishinari, 1998; Nickerson et al., 2003; Tang, Tung, & Zeng, 1998; Tang et al., 1995). The other is the change in anionic nature of the gellan chain as determined by the degree of dissociation of the carboxyl groups, which varies with pH (Horinaka et al., 2004). Acidification with glucono-δ-lactone (GDL) can produce a slow reduction in pH, depending on the solution temperature (Lucey, Tamehana, Singh, & Munro, 1998) and leading to gel properties different from those obtained using direct acidification. The use of GDL has been widely studied for milk protein gelation (Lucey & Singh, 1998) as it is easier to control the process acidification and hence the rheological properties or texture of the gels.

In this work, we investigated the influence of biopolymer concentration, final pH and the heating conditions on the rheological properties of gellan gels acidified by the addition of GDL. Oscillatory rheological measurements were performed to describe the gelation kinetics, and the gels were also characterized at the final pH with respect to their mechanical properties, water-holding capacity (WHC), turbidity and microstructure using scanning electron microscopy (SEM).

2. Materials and methods

2.1. Material

Deacylated gellan gum powder (Kelcogel® F) was kindly provided by Kelco Biopolymers (San Diego, CA), and was used without further purification. The gellan gum powder had a moisture content of approximately 10% (w/w) and the following ion composition (%, w/w): calcium (0.4), sodium (0.3), magnesium (0.1), potassium (4.9) and phosphorous (0.2). GDL was purchased from Sigma–Aldrich Corporation (St. Louis, USA).

2.2. Solution preparation and gel formation

Five different systems were analysed considering the heating conditions of the biopolymer solutions: not heated; heated (70 or 90 °C/30 min) before the addition of GDL and heated (70 or 90 °C/30 min) after the pH had reached the final value. For all the systems, the gellan gum powder, at different concentrations (0.2, 0.5, 1.0%, w/w), was dispersed in deionised distilled water at room temperature (25 °C) using a magnetic stirrer (original solution). Different amounts of GDL were used to decrease the pH to values ranging from 2 to 4 after storage at 25 °C for 48 h, though the addition of this acid precursor was done under different conditions. Measurement of the pH was carried out using a penetration glass electrode at different points of the gel, the readings becoming stable after approximately 5–10 min. The GDL/gellan ratio used for each biopolymer concentration is shown in Table 1. For the first condition studied, the GDL was added immediately after gellan gum dissolution and the resulting solution was stored at 25 °C for 48 h before the properties were measured. For the second and third conditions, the original solution was heated at 70 or 90 °C for 30 min. After the heat treatment, the solutions were cooled to 25 °C. The GDL was then added and the subsequent procedures were analogous to those performed for the first condition. The solutions heated after acidification (fourth and fifth conditions) were prepared in a similar way to the non-heated solutions. After the samples had reached the final pH (after storage at 25 °C for 48 h), they were heated at 70 or 90 °C for 30 min and cooled to room temperature before the physical properties were evaluated. Immediately after the addition of GDL, the biopolymeric solutions were subjected to oscillatory rheological measurements. After storage for 48 h, the gels formed were subjected to the following tests: uniaxial compression, WHC, turbidimetry and SEM. Turbidimetry and the oscillatory rheological measurements were only performed for the non-heated solutions and those heated before acidification. All measurements were done in triplicate.

Table 1
[GDL/gellan] ratio used to attain different pH values, as a function of biopolymer concentration

Gellan concentration (w/w)	pH				
	4.0	3.5	3.0	2.5	2.0
1%	0.1	0.25	0.95	3.7	15.0
0.5%	0.05[a]	0.25	1.2	5.0	16.0
0.2%	0.03[a]	0.1[a]	1.3[a]	9.0	55.0

[a] Samples did not form self-supporting gels after 48 h and were not evaluated.

2.3. Oscillatory rheology

Non-heated gellan gum solutions and those heated before acidification were poured directly onto the rheometer plate. Oscillatory shear measurements were performed using a stress-controlled rheometer (Carri- Med CSL^2 500, TA Instruments, UK). An acrylic cone and plate geometry (cone angle 0.07 rad; 40 mm in diameter) system was used to evaluate 1% (w/w) solutions. For solutions at lower concentrations, a concentric cylinder system (acrylic bob diameter 40 mm, cup diameter 42.5 mm) was used. Time sweeps were done at 0.1 Hz, 25 °C and 1.0 Pa for 1% solutions and at 0.5 Pa for the other samples. The gel point was determined using the criterion of the intersection between G' (storage modulus) and G'' (loss modulus) since this is the most used criterion for polysaccharide gels. The Lissajous figures were plotted to ensure that the measurements for G' and G'' were always within the linear viscoelastic region.

2.4. Uniaxial compression

The gels were formed in cylindrical plastic tubes (20 mm diameter, 20 mm height) and the uniaxial compression measurements were carried out using a TA-XTIIi Texture Analyser (Stable Micro Systems, UK) with a 40 mm diameter cylindrical acrylic plate lubricated with silicon oil to minimize friction between the sample and geometry. The mechanical properties at rupture were obtained by compressing the gels to 80% of their original height at 25 °C using a cross-head speed of 1 mm/s.

Hencky stress (σ_H) and strain (ε_H) were calculated from the force-deformation data according to Eqs. (1) and (2), respectively

$$\sigma_H = F(t) \cdot \frac{H(t)}{(H_0 \cdot A_0)}, \quad (1)$$

$$\varepsilon_H = \ln\left(\frac{H(t)}{H_0}\right), \quad (2)$$

where $F(t)$ is the force at time t, A_0 and H_0 are the initial area and height of the sample, respectively, and $H(t)$ is the height at time t.

The rupture properties were associated with the maximum point of the stress–strain curve. Stress (σ_{rup}) at rupture was used as an indicator of the gel hardness or strength and the strain (ε_{rup}) at rupture as an indicator of gel deformability.

2.5. Determination of the Water-Holding Capacity (WHC)

Samples of approximately 1 g were centrifuged at 124g for 10 min at 25 °C (Allegra 25 – R, A-10 rotor, Beckman Coulter, Germany). The centrifugation conditions used represented an adaptation of the method of Ikeda and Foegeding (1999). The water holding capacity of the gels was calculated from Eq. (3) and was expressed as a percentage.

$$WHC = 100 \cdot \left[1 - \left(\frac{\text{water}_{\text{released}}\ (\text{g})}{\text{water}_{\text{gel}}\ (\text{g})}\right)\right], \quad (3)$$

where $\text{water}_{\text{released}}$ is the amount of water released after centrifugation and $\text{water}_{\text{gel}}$ is the initial amount of water in the sample.

2.6. Turbidimetry

Gellan solutions were prepared as previously described, poured into cylindrical cuvettes (Beckman, Germany) and stored for 48 h at 25 °C. The light absorbance of the gellan gels was measured at $\lambda = 490$ nm with a spectrophotometer (Beckman Du-70 Spectrophotometer, Germany) using water as the reference.

2.7. Scanning electron microscopy (SEM)

Samples (~5 mm × 5 mm × 3 mm) of gels were fixed overnight in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2), in order to minimize structure modification during the posterior drying treatment. The fixed samples were fractured under liquid nitrogen and rinsed two times with cacodylate buffer. The samples were then dehydrated in a graded ethanolic series (20%, 40%, 60%, 70% and 90%). Dehydration was continued in 100% ethanol (three changes over 1 h) followed by critical point drying (Balzers Critical Point Dryer CPD03). The dried samples were mounted on aluminium stubs and coated with gold in a Balzers Sputter Coater SCD 050. At least three images of the typical structures at a magnification of 500 or 1000× were recorded, using a JEOL JSM 5800 LV (Tokyo. Japan) operated at 10 kV.

3. Results

3.1. Gelation kinetics

Using the criterion of crossover between G' and G'', the gel point could be observed for all samples that formed a self-supporting gel at the final pH. Although 1% gellan solutions at pH values between 2.5 and 2.0 formed self-supporting gels, an intersection point could not be observed, since G' was already greater than G'' from the beginning of the measurement due to the very high acidification rate used under these conditions. Table 2 shows the time (t_g) necessary to reach the gel point of the samples analysed. As can be observed from Table 1, solutions with the same biopolymer concentration required greater amounts of GDL to reach the lower pH values. Since the time taken to reach the final pH was the same for all systems, this meant that the acidification rate was enhanced by increasing the amount of GDL in solutions of the same gellan concentration, possibly explaining why the gel point could not be detected for 1% gellan systems between pH 2.5 and pH 2.0. On the other hand, the acidification rate decreased with decreasing biopolymer concentration at the same final

Table 2
Time to reach the gel point (t_g) of gellan gum solutions prepared with different concentrations of GDL and different heating conditions

pH	Heat	t_g (s)		
		Conc.		
		1%	0.5%	0.2%
4.0	n.a.	3848 ± 215^{a}	–	–
	70 °C	449 ± 18^{b}	–	–
	90 °C	676 ± 23^{c}	–	–
3.5	n.a.	1403 ± 93^{d}	$26{,}232 \pm 100^{a}$	–
	70 °C	343 ± 25^{e}	9336 ± 1184^{b}	–
	90 °C	393 ± 0^{e}	$37{,}152 \pm 746^{c}$	–
3.0	n.a.	536 ± 21^{f}	6967 ± 220^{d}	–
	70 °C	168 ± 0^{g}	6146 ± 429^{e}	–
	90 °C	175 ± 4^{g}	$11{,}479 \pm 647^{f}$	–
2.5	n.a.	–	1906 ± 21^{g}	6666 ± 6^{a}
	70 °C	–	2785 ± 164^{h}	5921 ± 42^{b}
	90 °C	–	3328 ± 69^{h}	5959 ± 245^{b}
2.0	n.a.	–	917 ± 38^{j}	906 ± 32^{c}
	70 °C	–	1051 ± 95^{j}	775 ± 0^{d}
	90 °C	–	1287 ± 215^{j}	790 ± 6^{d}

Means with the same letter in each column are not significantly different at $p < 0.05$. Conc., gellan concentration (w/w); Heat, heating conditions; n.a., no heating.

pH, which could explain the observation of a gel point in systems of lower concentration (0.5 and 0.2%) at pH values between 2.5 and 2.0. However, the amount of GDL necessary to reach a determined final pH was dependent on the gellan concentration. To reach higher final pH values, it was necessary to decrease the amount of GDL with decreasing polysaccharide concentration, but this trend was reverted for lower pH values (Table 1).

From Table 2 it can be seen that the time interval (t_g) required to reach gel point tended to decrease with increasing polymer and GDL concentrations, but the effect of heating was not clear. Samples heated at 90 °C showed higher t_g values than those heated at 70 °C, which was only observed at the higher equilibrium pH values. These pH values were different, depending on the biopolymer concentration: pH 4 for 1%, pH of 3.5 and 3 for 0.5% and no differences were observed for the heating conditions at 0.2%.

Fig. 1A–C shows the plots of G^* *versus* t/t_g for each concentration of the solutions. The decrease in the time taken to reach the gel point can be better visualized from the fast evolution of the complex shear modulus G^* as a function of the reduced time t/t_g. The use of t/t_g instead of t allows one to eliminate the effect of the kinetics of the gelation process. For systems with polymer concentrations of 0.5% and 0.2%, the divergence of G^* (Christ, Takeuchi, & Cunha, 2005) occurred at a value for t/t_g equal or very close to 1.0 (maximum value of 1.05) which means that the effective aggregation of the molecules began near the cross-over of G' and G''. Therefore, both criteria showed similar results and were appropriate for characterizing the gel point of gellan solutions at these concentrations.

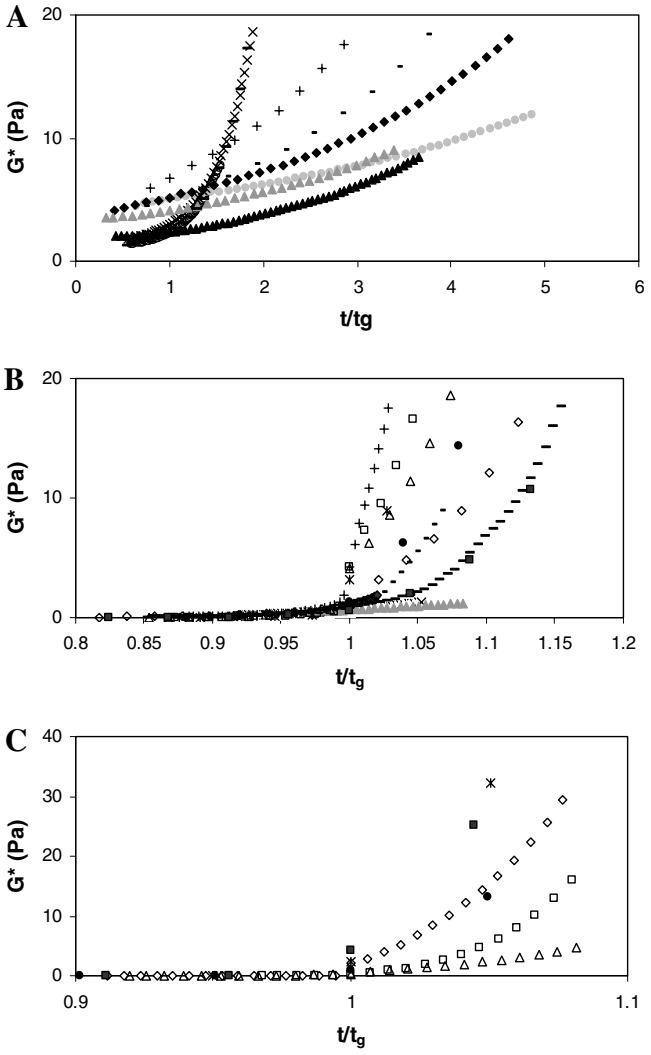

Fig. 1. Evolution of G^* as a function of t/t_g for 1% (A), 0.5% (B) and 0.2% (C) gellan gum solutions. n.a.: no heating. (○) pH 4.0, n.a.; (●) pH 4.0, 70 °C; (▲) pH 4.0, 90 °C; (×) pH 3.5, n.a.; (♦) pH 3.5, 70 °C; (▲) pH 3.5, 90 °C; (–) pH 3.0, n.a.; (+) pH 3.0, 70 °C; (-) pH 3.0, 90 °C; (◇) pH 2.5, n.a.; (□) pH 2.5, 70 °C; (△) pH 2.5, 90 °C; (■) pH 2.0, n.a., (*) pH 2.0, 70 °C; (●) pH 2.0, 90 °C.

On the other hand, for 1% systems two patterns of gelation could be distinguished, one with the highest reaction rate constituted of solutions that were not heated at pH values of 4.0, 3.5 and 3.0, and the other with a lower reaction rate comprising the heat-treated solutions. Fig. 1A shows that in heated solutions the value of t/t_g at which G^* diverges becomes greater. This means that, in fact, the aggregation process of the gellan molecules began at a point distant from the gel point as determined by the cross-over G'–G'' criterion. This was probably associated with the fact that entanglements among the molecules were favoured at high polymer concentrations, leading to a higher increase in G' with time, and not necessarily to junction zones formation. Therefore for 1% systems the intersection between G' and G'' may not be suitable for determining the gel point and the divergence of G^* or G' could be a more

adequate criterion to evaluate the sol–gel transition at higher gellan concentrations.

3.2. Mechanical properties, water holding capacity (WHC) and microstructure

None of the self-supporting gels, independent of polymer concentration, pH and heating conditions, showed syneresis. A similar result was previously reported for 1% gellan gels formed by adding calcium ions (Mao, Tang, & Swanson, 2001), which exhibited no sign of syneresis even after 60 days of storage. The absence of syneresis is an indication of the stability of the gel structure with respect to its WHC, and may represent a great advantage of gellan gels as compared to those of other polysaccharides such as carrageenan or locust bean gum.

Stress and strain at fracture and the WHC values are shown for the self-supported gellan gum gels in Fig. 2 and Tables 3 and 4, respectively. Such results were represented as a function of pH and heating conditions. Stress at fracture, which is related to gel strength (Zhang, Daubert, & Foegeding, 2005), tended to increase at higher polymer concentrations (Fig. 2). Similar behaviour was reported for gellan gels formed by cation addition (Miyoshi et al., 1995; Tang et al., 1994; Tang et al., 1998). The strength of the gels also increased by lowering the pH. However, 1% gellan gels only showed this behaviour until they reached pH 2.5, a decrease in failure stress occurring at pH 2.0, which did not occur for the gels of lower concentrations. The results obtained in this study for the strength of the gels in relation to pH for gellan gum gels acidifed by adding GDL are quite different from those reported in the literature for gels formed by direct acidification with acids like HCl and acetic acid (Mao, Tang, & Swanson, 1999; Moritaka et al., 1995; Moritaka, Naito, Nishinari, Ishihara, & Fukuba, 1999; Sanderson, 1990). In these works the failure stress of the gels decreased with decreasing pH, contrary to that occurring with the gellan gels formed from slower acidification by adding GDL, whose strength was continuously enhanced at the lower pH values (except for 1% gels at pH 2.0). Therefore a slow, controlled acidification with GDL can represent an alternative to obtain gellan gum gels with specific and differentiated texture characteristics, mainly under very acidic conditions, which cannot be attained by direct acidification (Mao et al., 1999; Moritaka et al., 1995; Moritaka et al., 1999; Sanderson, 1990). In addition, the strength of the gels was enhanced by heating the solutions before adding the GDL, but diminished by heating the solutions after acidification, in comparison to non-heated solutions. In all cases the heating temperature did not exert a major effect on this property.

In general, the strain at rupture values rose with increasing concentration and decreasing pH, except for 1% gellan at pH 2.0 and 0.5% at pH values of 3 and 2.5 (Table 3). In the latter case this unlikely behaviour was observed only for solutions heated before acidification. Moreover, the deformability of the 1% and 0.5% polysaccharide gels tended to decrease when heating was done before acidification (except 0.5% at pH 2.0), and to be higher for solutions heated at 70 °C after reaching the equilibrium pH, in comparison to non-heated solutions.

The WHC increased with increasing polymer concentration (Table 4) and tended to decrease with lowering of the pH for 1% and 0.5% gellan, but showed similar values between pH 3.0 and 2.0 for samples heated before acidification at the highest gellan concentration. Neither the application of heating nor the temperature of such a process predominantly affected the WHC of these gels. Nevertheless solutions heated before the addition of GDL, formed gels with slightly higher WHC under most conditions. For 0.2% gels this tendency was inverted, and the WHC increased with decreasing pH value (except when heated at 90 °C after acidification) and also tended to be higher for gels formed from solutions heated before acidification.

Figs. 3–5 show, respectively, the effects of polymer concentration, pH and heating on the microstructure of some of the gels analysed. Despite the differences in structure caused by variations in the process parameters, all showed a homogeneous pore distribution. Fig. 3 shows clearly that, by progressively diminishing the polymer concentration, the gel structure became less homogeneous and showed larger pores. The pores corresponded to the water entrapped in the gel network, which was evaporated during sample preparation. It can be seen in Figs. 4A-C that a continuous decrease in pH to 2.5 led to gels with a more densely linked structure and smaller pores, which was reflected in higher values of failure stress. Fig. 4D shows that 1% gellan gels at pH 2.0 had a more elongated structure with clear defects in their interior, which would be responsible for the diminished strength of these gels. Fig. 5 shows that the heating of solutions before the addition of GDL led to gels with a more uniform and densely linked structure than those formed from non-heated solutions. On the other hand, heating the gels after acidification resulted in a less homogeneous structure than the non-heated samples, which could explain the decreased failure stress of gels formed under this condition. However, it is interesting to observe that the microstructures in Fig. 5A and C are similar in the same way as for failure strain. In this case, the more porous structure could be reflecting greater deformability of the gels.

3.3. Turbidimetry

Gellan gels were visually transparent under all the conditions studied, even at pH 2.0, contrary to that reported for gellan gels formed by direct acidification with HCl, which were turbid at the same pH values (Moritaka et al., 1995). Thus absorbance measurements may be a very interesting and useful way to analyse the characteristics of the gel formed, since lower absorbance values represent

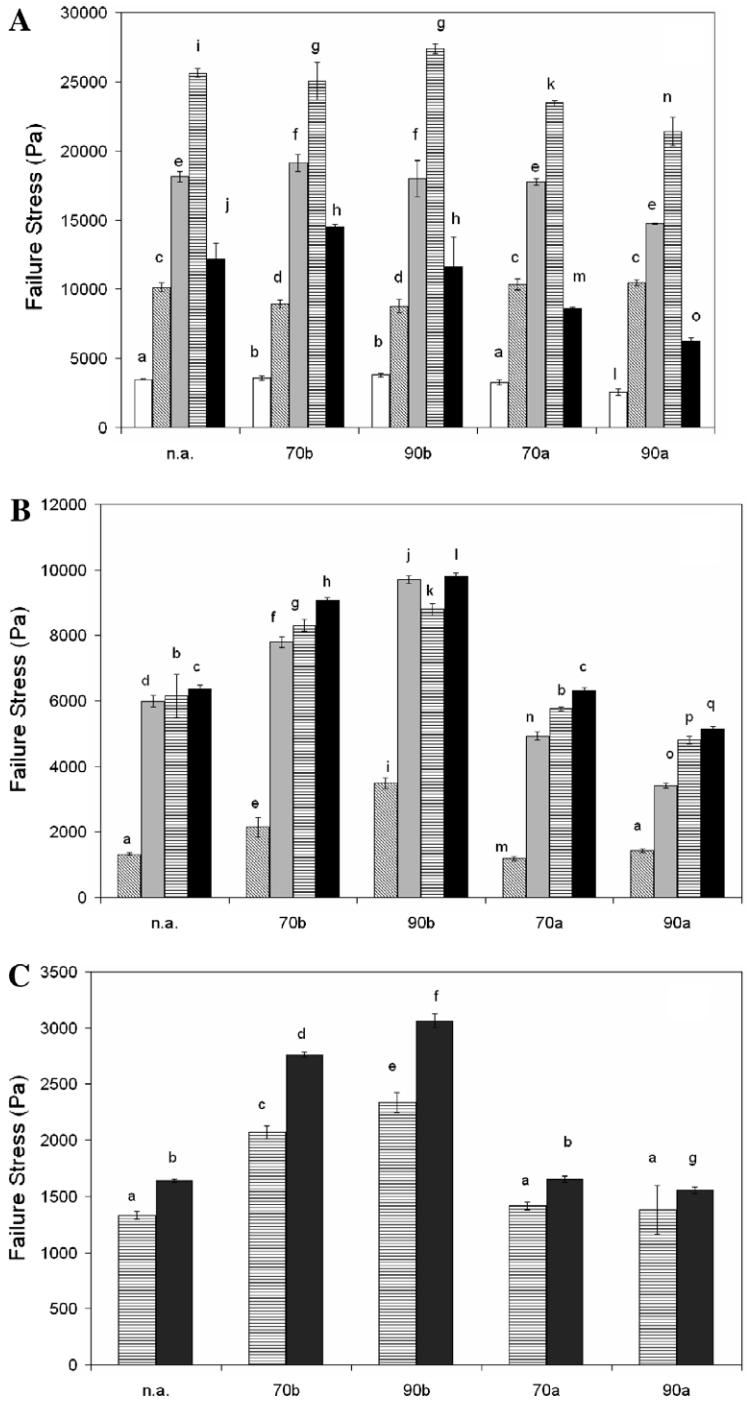

Fig. 2. Failure stress for 1% (A), 0.5% (B) and 0.2% (C) gellan gum gels. Bars with identical letter are not significantly different at $p < 0.05$ for each pH. n.a.: no heating; 70/90b: heating (70 or 90 °C/30 min) before acidification; 70/90a: heating (70 or 90 °C/30 min) after acidification. (□) pH 4.0; (▨) pH 3.5; (▩) pH 3.0; (▤) pH 2.5; (■) pH 2.0.

little turbidity and higher clarity of the gels (Tang, Mao, Tung, & Swanson, 2001). Fig. 6 shows the results for turbidity measurements as a function of pH and heating (or otherwise) for the gellan gels. Independent of the pH and heating conditions, absorbance, and consequently the turbidity of the solutions, increased with increasing polymer concentration. Such an increase led to a thicker structure which produced greater light scattering and consequently, greater turbidity. This same behaviour had already been observed for gellan gels formed by adding calcium ions (Tang et al., 2001).

At each concentration, solutions heated before acidification tended to form less turbid gels, independent of the heating temperature, with the exception of the 1% solutions at pH values from 2.5 to 3.5, which exhibited lower values of absorbance for the non-heated solutions. Decreasing pH also led to the formation of gels with higher absorbance values, as can be seen in Fig. 6. Nevertheless, the

Table 3
Failure strain of gellan gum gels prepared with different concentrations of polysaccharide and different heating conditions

Conc.	Heat	Failure strain (dimensionless)				
		pH				
		4.0	3.5	3.0	2.5	2.0
1%	n.a.	0.35 ± 0.01^{a}	0.36 ± 0.00^{e}	0.36 ± 0.02^{g}	0.40 ± 0.02^{i}	0.25 ± 0.02^{n}
	70b	0.20 ± 0.02^{b}	0.24 ± 0.01^{f}	0.21 ± 0.04^{h}	0.35 ± 0.03^{j}	0.22 ± 0.01^{n}
	90b	0.26 ± 0.02^{c}	0.24 ± 0.03^{f}	0.27 ± 0.00^{h}	0.32 ± 0.01^{k}	0.23 ± 0.01^{n}
	70a	0.38 ± 0.06^{a}	0.39 ± 0.02^{e}	0.38 ± 0.01^{g}	0.43 ± 0.01^{l}	0.29 ± 0.03^{n}
	90a	0.24 ± 0.02^{d}	0.37 ± 0.03^{e}	0.39 ± 0.01^{g}	0.43 ± 0.01^{m}	0.26 ± 0.05^{n}
0.5%	n.a.	–	0.24 ± 0.02^{a}	0.35 ± 0.01^{c}	0.33 ± 0.00^{h}	0.31 ± 0.00^{l}
	70b	–	0.19 ± 0.05^{b}	0.29 ± 0.01^{d}	0.26 ± 0.01^{i}	0.30 ± 0.01^{l}
	90b	–	0.18 ± 0.01^{b}	0.35 ± 0.02^{e}	0.25 ± 0.02^{j}	0.34 ± 0.02^{l}
	70a	–	0.24 ± 0.02^{a}	0.38 ± 0.01^{f}	0.32 ± 0.01^{h}	0.30 ± 0.01^{l}
	90a	–	0.26 ± 0.03^{a}	0.30 ± 0.02^{g}	0.28 ± 0.01^{k}	0.27 ± 0.00^{m}
0.2%	n.a.	–	–	–	0.26 ± 0.01^{a}	0.28 ± 0.00^{c}
	70b	–	–	–	0.33 ± 0.01^{b}	0.36 ± 0.01^{d}
	90b	–	–	–	0.33 ± 0.01^{b}	0.40 ± 0.01^{e}
	70a	–	–	–	0.27 ± 0.02^{a}	0.28 ± 0.00^{c}
	90a	–	–	–	0.27 ± 0.03^{a}	0.25 ± 0.00^{f}

Means with the same letter in each column are not significantly different at $p < 0.05$ for each pH. Conc., gellan concentration (w/w); ann., heating; n.a., no heating; 70/90b, heating (70 or 90 °C/30 min) before acidification; 70/90a, heating (70 or 90 °C/30 min) after acidification.

Table 4
WHC of gellan gum gels prepared with different concentrations of polysaccharide and different heating conditions

Conc.	Heat	WHC (%)				
		pH				
		4.0	3.5	3.0	2.5	2.0
1%	n.a.	85.97 ± 3.51^{a}	83.41 ± 0.45^{c}	79.42 ± 1.19^{g}	77.03 ± 0.77^{j}	75.94 ± 2.28^{l}
	70b	90.40 ± 1.47^{b}	86.15 ± 1.10^{d}	81.58 ± 1.18^{h}	80.90 ± 1.80^{k}	81.41 ± 0.76^{m}
	90b	90.63 ± 3.79^{b}	89.54 ± 0.84^{e}	83.06 ± 1.64^{h}	82.08 ± 0.54^{k}	82.82 ± 1.17^{m}
	70a	82.75 ± 2.13^{a}	84.30 ± 2.78^{c}	79.60 ± 1.22^{g}	74.81 ± 2.95^{j}	72.30 ± 0.82^{l}
	90a	84.13 ± 1.08^{a}	86.61 ± 1.44^{f}	82.65 ± 1.42^{i}	78.18 ± 1.26^{j}	72.42 ± 0.63^{l}
0.5%	n.a.	–	78.01 ± 0.51^{a}	64.94 ± 0.57^{d}	44.13 ± 0.83^{i}	35.12 ± 6.23^{k}
	70b	–	76.75 ± 0.11^{b}	66.77 ± 0.70^{e}	44.11 ± 3.34^{i}	31.73 ± 4.27^{l}
	90b	–	75.75 ± 4.30^{c}	69.63 ± 0.91^{f}	45.31 ± 1.23^{i}	33.74 ± 3.33^{l}
	70a	–	75.36 ± 1.60^{a}	61.63 ± 0.12^{g}	44.84 ± 7.16^{i}	43.98 ± 0.37^{m}
	90a	–	77.96 ± 0.24^{a}	57.53 ± 1.23^{h}	38.46 ± 1.37^{j}	25.25 ± 8.18^{n}
0.2%	n.a.	–	–	–	15.33 ± 1.60^{a}	21.56 ± 1.74^{c}
	70b	–	–	–	19.80 ± 1.10^{b}	31.70 ± 1.68^{d}
	90b	–	–	–	19.06 ± 3.50^{b}	32.20 ± 1.17^{d}
	70a	–	–	–	15.19 ± 1.95^{a}	16.46 ± 1.40^{e}
	90a	–	–	–	12.62 ± 1.86^{a}	9.31 ± 0.22^{f}

Means with the same letter in each column are not significantly different at $p < 0.05$ for each pH. Conc., gellan concentration (w/w%); ann., heating; n.a., no heating; 70/90b, heating (70 or 90 °C/30 min) before acidification; 70/90a, heating (70 or 90 °C/30 min) after acidification.

occurrence of a point of maximum absorbance for 1% (pH 3.0) and 0.5% (pH 2.5) gellan gels is worthy of note. In addition, the lower the polymer concentration the lower the pH at which the point of maximum value occurred, and therefore it is possible that if pH values below 2.0 had been studied, a point of maximum absorbance might also have been observed for the 0.2% gels. A peak value for the mechanical properties was also observed for 1% gels, but at pH 2.5.

Since an increase in turbidity values corresponds to a structure with higher cross-linking density, absorbance measurements should be directly related to gel strength. In fact, in a general way, a direct correlation was observed between these two parameters (data not shown), which means that a higher turbidity value corresponded to a stronger gel.

4. Discussion

At higher concentrations, the gellan chains are closer to each other, enhancing the probability of aggregation and the formation of junction zones. This led to a decrease in the time to reach gel point and a more densely linked network structure formation with thicker strands (Fig. 3), which corresponded to harder and turbid gels. Moreover a densely linked structure probably allowed for a more

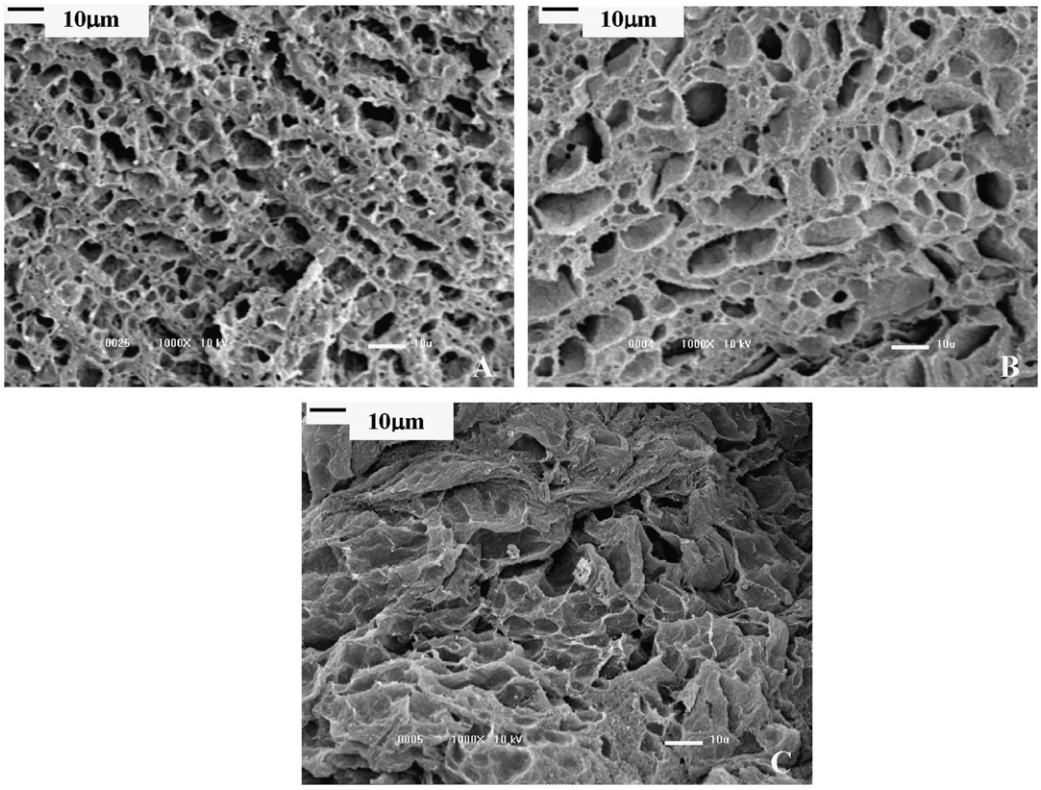

Fig. 3. SEM micrographs of 1% (A), 0.5% (B) and 0.2% (C) gellan gels at pH 2.5 formed from non-heated solutions.

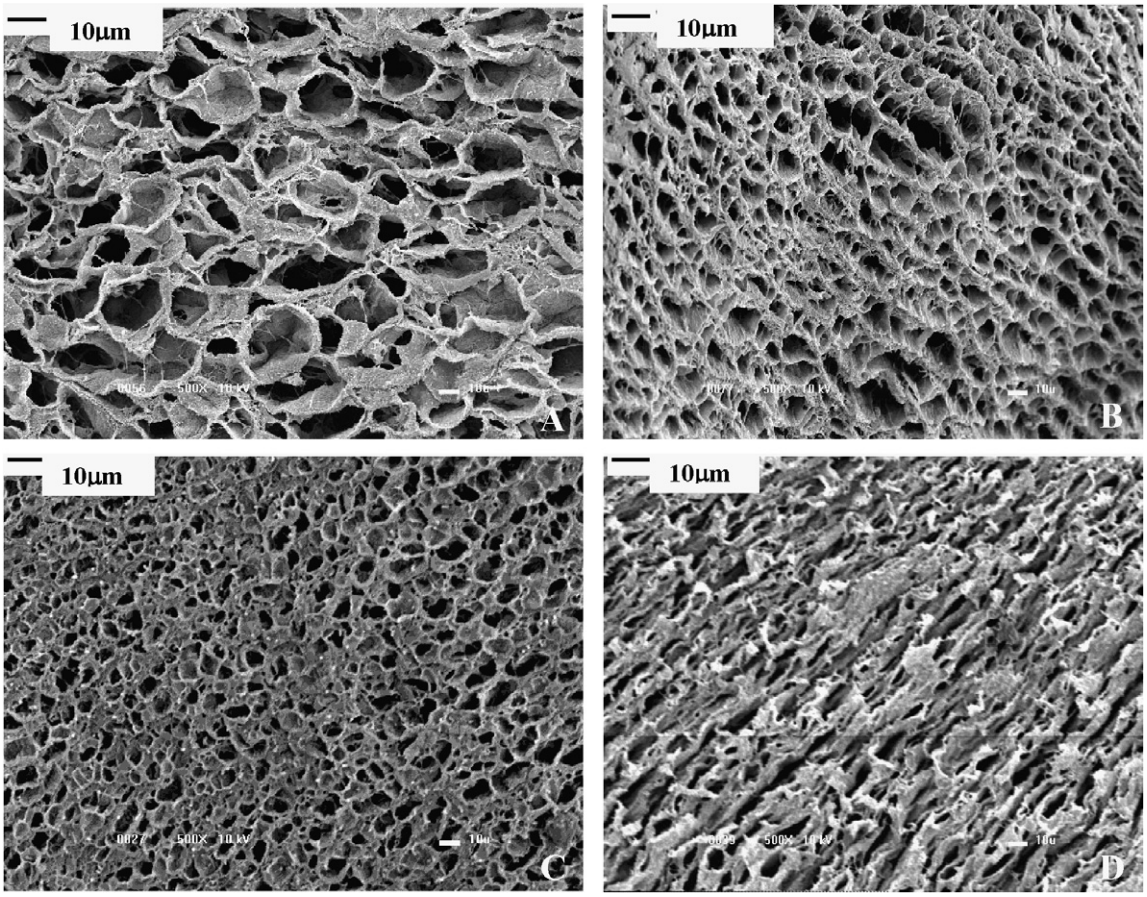

Fig. 4. SEM micrographs of 1% gellan gels heated at 70 °C/30 min before acidification at final pH values of 4.0 (A), 3.0 (B), 2.5 (C) and 2.0 (D).

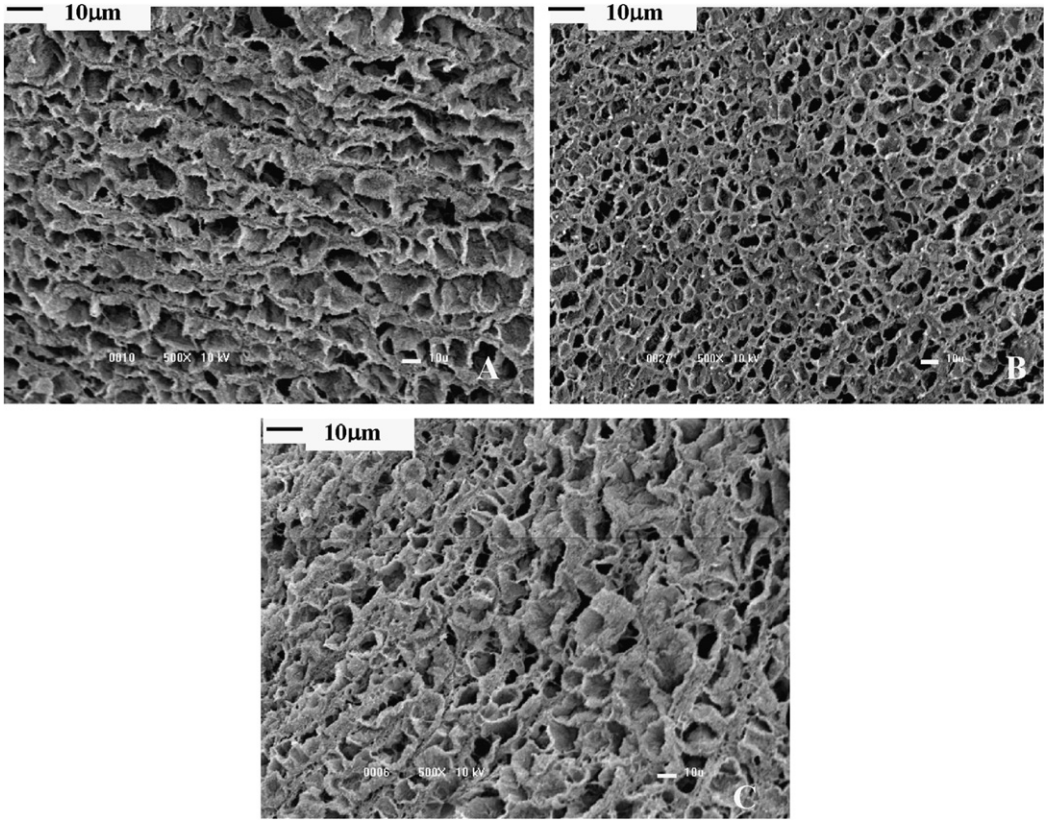

Fig. 5. SEM micrographs of 1% gellan gels formed from non-heated solutions (A), solutions heated at 70 °C/30 min before acidification (B) and solutions heated at 70 °C/30 min after acidification (C).

uniform distribution of the applied stress and therefore the failure strain or deformability was higher for more concentrated gels. The matrix also tended to have smaller pores and a higher number of junction zones, producing stronger networks. It is harder for such structures to collapse, even with the application of external forces, leading to increased WHC values.

The effect of pH on gellan gelation could be associated with the lower charge density of chains at low pH values (Horinaka et al., 2004). Since the carboxyl group included in the gellan chain is a weak acid group, and 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 to be expected that the less anionic chains aggregate with one another more easily because of the lower electrostatic repulsion. In addition, the decrease in electrostatic repulsion between the intramolecular segments may result in the suppression of gellan chain expansion, making association even easier. The ease of aggregation caused by decreasing the pH explains the decrease in time to reach gel point, the more densely linked structure at equilibrium (Fig. 4) and consequent increases in gel strength, deformability and turbidity. On the other hand, the decrease in the number of dissociated carboxyl groups also led to a decrease in the amount of bound water, expressed as a lower WHC value. In the case of 0.2% gels, the amount of molecules was significantly lower than in the other systems studied (0.5% and 1%). As a consequence, the decrease in anionic sites able to link water did not predominantly influence the ratio between bound and free water. In this case the pH effect seemed to be associated with the increase in aggregation caused by lowering the pH. Since a more densely linked structure tends to have smaller pores, the capillary forces that maintained water inside the structure increased and therefore the WHC of the system also increased.

Decreases in temperature induced the conformational transition of gellan chains from helixes to random coils. Since the solutions were cooled to room temperature (25 °C) before the addition of GDL, the molecules probably returned to their helical form. Nevertheless, heating gellan solutions at temperatures above its gel–sol transition before acidification appeared to alter the degree of energy of the molecules, forming potential reaction sites and increasing the size and stability of the gellan strands (Yoshida & Takahashi, 1993). These strands formed large aggregates, which promoted a decrease in the time to reach gel point and increase in gel strength. On the other hand, the structure formed in the heated gels showed a more homogeneous distribution of smaller pores (Fig. 5), making light diffraction through the matrix easier and decreasing gel turbidity. This means that gels formed from solutions heated before acidification were clearer, except

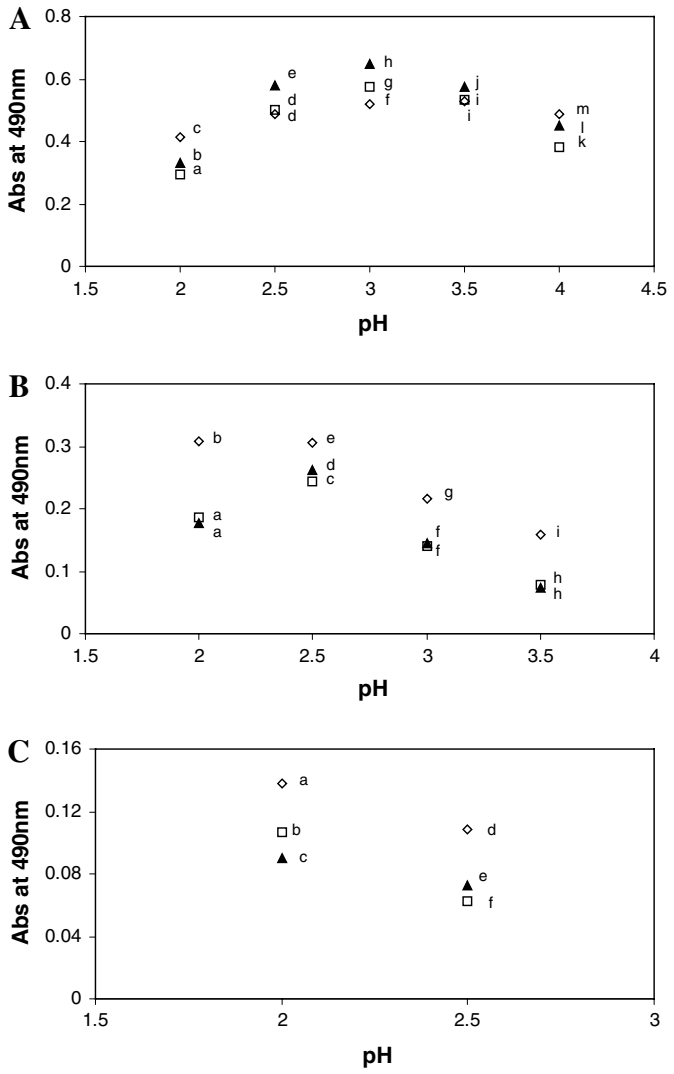

Fig. 6. Absorbance at 490 nm as a function of pH and heating for 1% (A), 0.5% (B) and 0.2% (C) gellan gels. Symbols with identical letters are not significantly different at $p < 0.05$. (◇) no heating; (□) heating (70 °C/30 min) before acidification; (▲) heating (90 °C/30 min) before acidification.

for the 1% gellan gels that showed higher absorbance at pH values from 2.5 to 3.5. In spite of the fact that heating allowed for the formation of a more homogeneous structure such a process appeared not to exert a strong influence on WHC. The water molecules are mainly in their free form in gellan gels formed by adding cations (Ohtsuka & Watanabe, 1996; Tang et al., 1998) and this also seems to be the case for gellan gels formed by acidification with GDL. In this way, independent of the heating conditions, water would continue to be mainly in the free form. However, it can be noticed that the WHC of gellan gels formed from solutions heated before GDL addition was slightly higher. Yoshida and Takahashi (1993) reported that the amount of bound water was increased by increased time and temperature of heating. Nevertheless, in our study the heating temperature only had a minor effect on the rheological properties and WHC of the gels.

The strength of gels that were heated after equilibrating the pH was diminished, as can be seen in Fig. 2. A possible explanation for this fact is that the interactions occurred during the previous process of acidification, and that some of these cross-links might not have been very thermostable and could have been broken during the heating process. For non-heated systems or those heated before acidification, these acid-induced aggregates were formed after cooling the solutions, resulting in stronger gels than those heated after acidification. Nevertheless, the gels did not recover their sol state after heating, demonstrating their thermo-irreversibility, similar to the systems formed by the addition of divalent cations but in a way opposite to those formed by the addition of monovalent ions, whose junction zones completely melted on heating to 50 °C (Miyoshi et al., 1995). This reinforces the fact that the effect of slow acidification with GDL on gel formation is significantly different from the shielding effect caused by the addition of cations (Horinaka et al., 2004).

Diminishing the pH led to enhanced turbidity and gel strength, but a peak of maximum value in the absorbance and mechanical properties was observed at the higher polysaccharide concentrations. At pH values below this point, local aggregation with the consequent formation of a weaker and less deformable structure could be occurring. Local aggregation of the molecules also resulted in a structure with larger pores through which the light passed more freely, and therefore the turbidity diminished. Nevertheless, for 1% gels the peak value of absorbance occurred at pH 3.0 whereas when the mechanical properties were considered it was only observed at pH 2.5. For 0.5% gels, a point of maximum absorbance was observed at pH 2.5, but no peak appeared in relation to their mechanical properties. Thus it could be concluded that the absorbance measurements were more sensitive with respect to observing structural changes than the mechanical properties, in a certain way foreseeing what would have occurred to the mechanical properties of the gels if the measurements had been done at lower pH values.

5. Conclusions

The results obtained allowed for the characterization of the rheological behaviour and microstructure of gellan gum gels formed by slow acidification with GDL. Acidification under these conditions constituted an effective way of obtaining strong, transparent gels, even, and in fact mainly, under very acidic conditions. It was also shown to be possible to manipulate the structure–texture of the system by the correct choice and combination of the gel formation conditions.

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