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Texture profile and turbidity of gellan/gelatin mixed gels

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Abstract

The effect of gellan (1.6–0.2%) to gelatin (0–1.4%) ratio and calcium ion concentration (0–30 mM) on the textural properties and turbidity of gellan/gelatin mixed gels was examined using instrumental Texture Profile Analysis (TPA) and spectrophotometry. Hardness of the mixed gels decreased as the proportion of gellan decreased. Hardness increased with increasing calcium ions until calcium concentration reached a critical level, after which further increases in calcium resulted in a reduction of hardness. Brittleness, springiness and cohesiveness were very sensitive to low levels of added calcium (0–10 mM), but less sensitive to higher calcium concentrations and gellan/gelatin ratio. In general, the addition of calcium ions caused gels to be more brittle and less cohesive and springy. Decreasing gellan to gelatin ratio caused an increase in gel turbidity at lower calcium ion levels (2–4 mM) and a decrease in turbidity at high calcium levels (20–30 mM). Maximum turbidity was observed in 0.6% gellan–1.0% gelatin gels without added calcium. The results of this study suggested a weak positive interaction between gellan and gelatin when no calcium was added, whereas at higher calcium levels gellan formed a continuous network and gelatin a discontinuous phase. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Gellan; Gelatin; Gel; Texture profile analysis; Turbidity

1. Introduction

Hydrocolloids offer many functional properties such as gelling, emulsifying, thickening, binding, suspending and coating (Anon., 1992). Of particular interest is the use of hydrocolloids for gelation, such as in the formation of water-based gels or as fat replacers in low-fat foods. Gellan is a bacterially derived polymer produced by a microbial fermentation process using the bacterium *Sphingomonas elodea* (formerly referred to as *Pseudomonas elodea*). High-acyl gellan, which forms only weak gels, is recovered directly from the fermentation broth, whereas low-acyl gellan is obtained by deacylation with alkali treatment (Sanderson, 1990) and forms strong gels with the proper formulation of polymer and cation concentrations. In this study, commercial low-acyl gellan was used.

The formation of gellan gels occurs in the presence of either monovalent or divalent cations by cooling hot polymer solutions (Sanderson, 1990). A two-step gelation mechanism has been proposed where double helices are initially formed from random coil chains followed by aggregation of pairs of double helices (Chandrasekaran & Radha, 1995). Divalent cations have a much greater effect on the gel properties than monovalent cations (Chandrasekaran & Radha; Sanderson & Clark, 1983; Tang, Lelievre, Tung & Zeng, 1994; Tang, Tung & Zeng, 1995). A relatively large range of textural properties can be obtained with gellan gels by properly selecting ionic strength and gellan concentration (Sanderson, 1990; Sanderson & Clark; Tang et al., 1994, 1995; Tang, Tung, Lelievre & Zeng, 1997; Tang, Tung & Zeng, 1996).

Gelatin gels are quite soft and flexible, but their textural properties, in general, are very narrow (Johnston-Banks, 1990; Munoz, Pangborn & Noble, 1986a,b; Wolf, Beach, La Velle & Clark, 1989). Compared to gellan gels, formation of gelatin gels requires a higher polymer concentration, a longer setting time and lower setting temperature (Wolf et al., 1989). The gel strength of gelatin gels is dependent on the gelatin concentration, with little effect of ionic strength and pH (Papageorgiou, Kasapis & Richardson, 1994; Hsu & Jamieson, 1993).

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Wolf et al. (1989) suggested that incorporation of small amounts of gellan into gelatin could greatly improve gelatin gel properties such as gelling and melting temperature, gel strength and room temperature stability. Morris (1990) indicated that combinations of gellan and gelatin may be useful in many food applications such as water or milk-based dessert gels, dessert syrups and toppings, fabricated fruits, vegetables, meat or fish aspics, and pet foods. Shim (1985) proposed that a synergistic increase in network strength can result by incorporation of gellan into gelatin. Sanderson (1990) reported that by adding 0.25% gellan to 250 Bloom type A gelatin, hardness and springiness of the resulting gels could be increased, and Wolf et al. (1989) showed that addition of gellan to gelatin gels can improve the firmness of the mixed gels. However, most studies on gellan/gelatin mixed gels have been limited to blends of small amounts of gellan (typically 0.05–0.5%) with larger amounts of gelatin.

Instrumental Texture Profile Analysis (TPA) has been used for many years for the measurement of food textural properties (Bourne, 1982; Sanderson, 1990). The textural parameters obtained from TPA force/deformation curves have been well correlated with sensory evaluation of textural parameters (Bourne; Mohsenin & Mittal, 1977; Munoz et al., 1986b), provides more information than "gel strength" measurements, and is useful for routine analysis of gel texture (Sanderson).

The objective of this study was to evaluate the effect of gellan to gelatin ratio (at a constant total polymer concentration) and calcium concentration on textural properties and clarity of gellan/gelatin mixed gels using instrumental TPA and spectrophotometry. The information from this study could be used to identify conditions for formulation of gels with specific characteristics, and to gain insight into potential interactions between the polymers under the test conditions.

2. Materials and methods

2.1. Preparation of gellan/gelatin mixed gels

Blends of gellan (Kelco Inc., San Diego, CA) and type B gelatin (from bovine skin, approximately 225 Bloom, Sigma Chemical Co., St. Louis, MO) at concentrations ranging from 0.2 to 1.6% (w/v) gellan with 1.4–0% (w/v) gelatin to make a total polymer concentration of 1.6%, were dispersed in deionized distilled water at room temperature with the aid of a magnetic stir bar. The solutions were heated with constant stirring to 90°C, and calcium chloride at concentrations ranging from 0 to 30 mM was added. The hot solutions were poured into glass tubes (30 mm diameter, 250 mm length) and cooled in running tap water for 15 min. Samples were held overnight at room temperature prior to texture measurements. Gellan powder contained about 0.4% (dry basis) calcium, 0.3% sodium and 4.9% potassium (data supplied by manufacturer), and gelatin powder contains about 0.055% calcium, 0.022% magnesium, 0.196% sodium and 0.016% potassium (USDA, 1998).

2.2. Instrumental TPA

The gels were removed from the glass tubes, cut into cylinders of 20 mm length and 30 mm diameter and then subjected to an instrumental texture profile analysis (TPA) similar to that described by Bourne (1982). The gel specimens were placed between parallel flat plate fixtures fitted to a TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) interfaced with a microcomputer. The specimens were lubricated with mineral oil on both ends prior to measurement, and all measurements were made on gels equilibrated to ambient temperature. The gels were compressed twice at 0.5 mm/s to 30% of their original height. The results are reported as the means of duplicate tests. Textural parameters such as hardness, brittleness, cohesiveness and springiness can be obtained from the TPA curve as shown in Fig. 1. The textural parameters considered in the present study (Bourne, 1982; Bourne & Comstock, 1981; Sanderson, 1990) were defined as:

- Hardness — the peak force during the first compression cycle.
- Brittleness — the first significant break during the first compression cycle divided by the original sample height, reported as a percentaage. Note that a small brittleness value indicates a more brittle gel.
- Cohesiveness — the ratio of the area under the first and second compression (A2/A1).
- Springiness — the distance the sample was compressed during the second compression to the peak

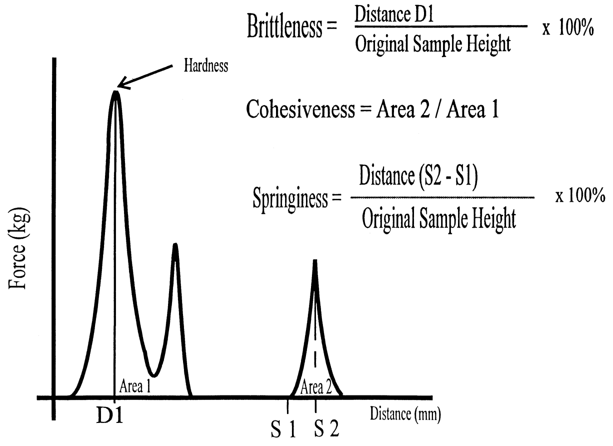

Fig. 1. Typical instrumental texture profile analysis curve for gellan/gelatin mixed gels.

force, divided by the initial sample height, reported as a percentage.

2.3. Turbidity measurements

Each hot polymer solution was poured into 1 cm plastic cuvettes and allowed to gel at room temperature. Samples were held overnight at ambient temperature prior to turbidity measurement. The turbidity of the gels was measured using a Pye Unicam PU 8600 UV/Visible Spectrophotometer (Pye Unicam Ltd., Cambridge, UK) at 550 nm against distilled water. Each turbidity measurement was carried out in duplicate.

2.4. Statistical analyses

Analysis of variance was used to examine the effects of gellan to gelatin ratio and calcium ion concentration on textural parameters and turbidity of gellan/gelatin mixed gels using Minitab 10.5 for Windows (Minitab Inc., State College, PA). Surfer 5.01 (Golden Software Inc., Golden, CO) was used to generate three-dimensional graphs.

3. Results and discussion

3.1. Textural analysis

3.1.1. Hardness

Hardness is related to the strength of the gel structure under compression. The effects of gellan to gelatin ratio and calcium concentration on gel hardness are shown in Fig. 2. In general, with addition of calcium ions, gel hardness increased to a maximum and then gradually decreased but the maximum hardness occurred at different calcium ion levels for different ratios of gellan and gelatin. Hardness also increased as the proportion of gellan increased. Chandrasekaran, Millane, Arnott and Atkins (1988) stated that the presence of cations would induce the gelation of gellan gum with the formation of linkages between the aggregated helices, divalent cations being effective at much lower concentrations than monovalent. The results of the present study suggest that there is a critical level of calcium ion concentration for hardness of the mixed gels, below which hardness increases and above which hardness decreases. Sanderson (1990) and Tang et al. (1994, 1995) observed a reduction in gel strength in gellan gels when calcium concentrations were above a critical value. Tang et al. (1994) suggested that excess calcium ions might occupy anionic sites of gellan gum molecules, thus preventing the formation of linkages between adjacent polymer chains. As a result, repulsive forces in the junction zones may reduce the formation of linkages between aggregated helices, leading to weakened gel structures. In the present study, this effect was most clearly evident at low gellan/gelatin ratios where saturation of potential carboxylate binding sites on the gellan polymer would occur at lower levels of calcium. Alternatively, Kasapis et al. (1999) suggested that excess calcium ions cause the formation of an inordinate number of ordered nuclei in the hot sol, which results in a low functionality network upon gelation.

3.1.2. Brittleness

The ionic strength and the ratio of gellan to gelatin significantly affected the brittleness of the mixed gels ($P < 0.05$). In general, the gels became more brittle (i.e. brittleness values got smaller) with increasing calcium ion concentration (Fig. 3). Gellan gels without addition of either gelatin or calcium were the least brittle of any of the gels. At high calcium levels, the mixed gels were very brittle (values less than 30%) but brittleness was

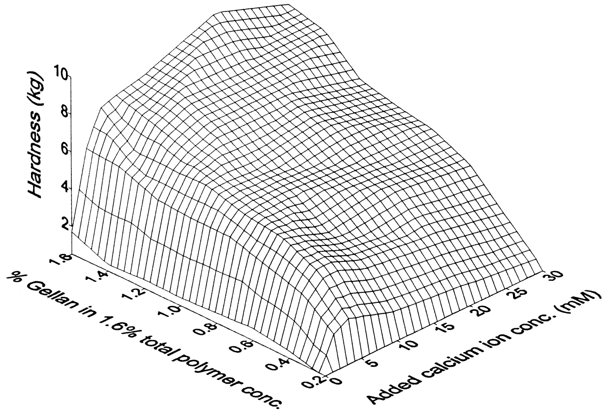

Fig. 2. Hardness of gellan/gelatin mixed gels as a function of gellan to gelatin ratio and added calcium ion level. The 1.6% total polymer solutions contained gellan (1.6–0.2% w/v) and gelatin (0.0–1.4% w/v). The axes have been arranged to best show the treatment effects on hardness.

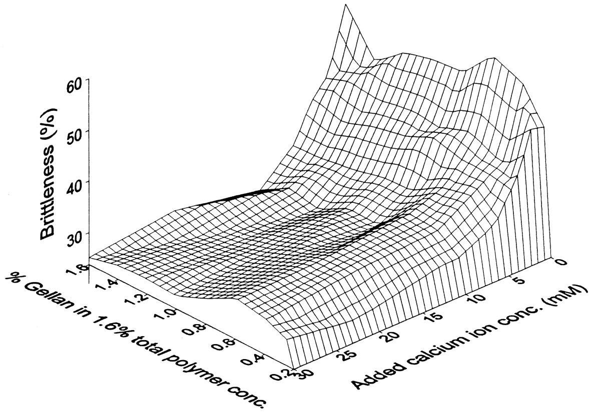

Fig. 3. Brittleness of gellan/gelatin mixed gels as a function of gellan to gelatin ratio and added calcium ion level. The 1.6% total polymer solutions contained gellan (1.6–0.2% w/v) and gelatin (0.0–1.4% w/v). The axes have been arranged to best show the treatment effects on brittleness.

also affected by the level of gelatin, where addition of gelatin, especially at higher levels of calcium, tended to make the gels less brittle. Overall, however, calcium influenced brittleness much more than gellan/gelatin ratio at levels of added calcium up to about 10–15 mM. Sanderson, Bell, Burgum, Clark and Ortega (1987) observed that combinations of gellan gum with non-gelling hydrocolloids such as xanthan gum, guar gum, locust bean gum, carboxymethycellulose and tamarind gum did not greatly affect brittleness of the mixed gels. However, Sanderson (1990) noted a gradual reduction in brittleness of gellan/gelatin mixed gels (calcium level was not reported) with increasing addition of gelatin to gellan gels, as did Wolf et al. (1989) who also reported that brittleness of gellan/gelatin mixed gels decreased with increasing gelatin level and suggested that gellan was predominant over gelatin as an influencing factor for brittleness. In the present study, it is likely that the gellan component in the mixed polymer systems was mainly responsible for the brittle characteristics of the mixed gels since even at the lowest ratio of gellan to gelatin, brittleness was highly dependent on calcium ion concentration. Moreover, the brittleness of the mixed gels showed similar trends to those reported by Tang et al. (1994, 1996, 1997) for the failure shear strain of gellan gels, where the gels could be deformed to a large extent at very low levels of divalent cation, but quickly decreased as cation level increased.

3.1.3. Cohesiveness

Cohesiveness is a measure of the degree of difficulty in breaking down the gel's internal structure (Sanderson, 1990; Wolf et al., 1989). There was a dramatic decrease in cohesiveness of the mixed gels when calcium ions were added (Fig. 4). Cohesiveness at any particular calcium concentration depended on the ratio of gellan to gelatin in the gel, but in general, the highest cohesiveness values appeared to be associated with either very high or very low gellan/gelatin ratios. These observations are similar to those reported by Wolf et al. when small amounts of gellan polymer were incorporated into gelatin. The results suggest that the internal structure of gels without added calcium may be more difficult to break during the first compression than the gels with the addition of calcium. Since most of the gel matrix was not broken during the first compression, more energy was required to break the remaining gel matrix during the second compression so that the gels may be perceived as being tough when chewed.

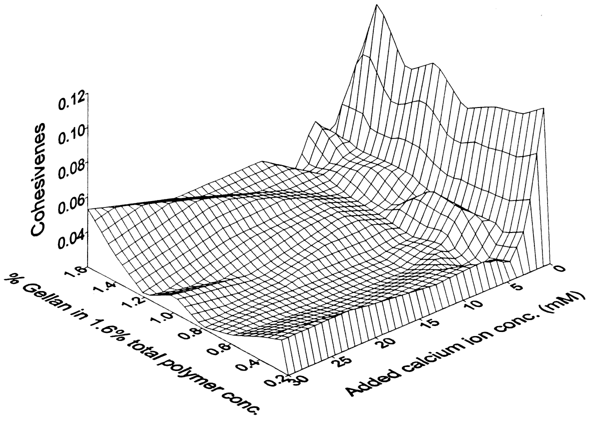

Fig. 4. Cohesiveness of gellan/gelatin mixed gels as a function of gellan to gelatin ratio and added calcium ion level. The 1.6% total polymer solutions contained gellan (1.6–0.2% w/v) and gelatin (0.0–1.4% w/v). The axes have been arranged to best show the treatment effects on cohesiveness.

3.1.4. Springiness

Springiness (sometimes also referred to as "elasticity") is a perception of gel "rubberiness" in the mouth, and is a measure of how much the gel structure is broken down by the initial compression (Sanderson, 1990). High springiness will result when the gel structure is broken into few large pieces during the first TPA compression whereas low springiness results from the gel breaking into many small pieces. Less springy gels, such as low-methoxy pectin, carrageenan and agar gels would break down more easily during mastication than a firm and springy gelatin gel (Marshall & Vaisey, 1972). Springiness was significantly ($P < 0.05$) affected by the addition of calcium and the gellan/gelatin ratio (Fig. 5). When a small amount of calcium ions was added, there was a rapid decrease in gel springiness followed by a more gradual increase and then decrease again as more calcium was added. Springiness was highest at a ratio of 0.4% gellan–1.2% gelatin without added calcium, and lowest at a combination of 0.8% gellan–0.8% gelatin and a high level of calcium.

3.1.5. Turbidity

The effects of gellan to gelatin ratio and calcium concentration on turbidity (Abs 550 nm) are shown in Fig. 6. Turbidity was significantly affected by both the calcium

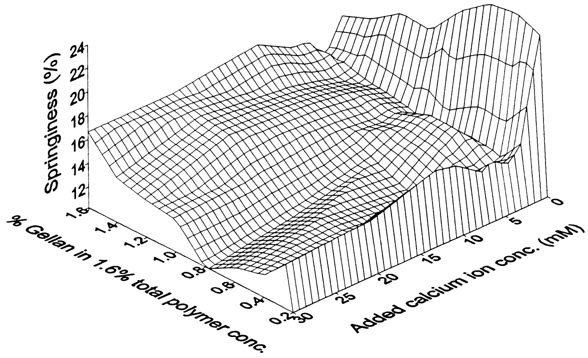

Fig. 5. Springiness of gellan/gelatin mixed gels as a function of gellan to gelatin ratio and added calcium ion level. The 1.6% total polymer solutions contained gellan (1.6–0.2% w/v) and gelatin (0.0–1.4% w/v). The axes have been arranged to best show the treatment effects on springiness.

ion concentration and gellan to gelatin ratio ($P < 0.05$). Clear gels were formed when gellan alone and low levels of calcium were used. In general, the mixed gels containing high calcium concentrations were more turbid than those with lower calcium concentration, except at lower levels of gellan. In the gels with gellan alone, turbidity increased with increasing calcium ion concentration. The increased turbidity is believed to be caused by the formation of light scattering aggregates upon cooling. Gelation of gellan is triggered by the addition of ions, which allows the helices of the polymeric strands to aggregate (Anon., 1992). Therefore, the addition of calcium ions would increase the aggregation of helices to form a three dimensional network, and as calcium concentration increased, extensive aggregation would be expected to increase the number and size of aggregates, with a subsequent increase in turbidity. At high calcium levels (20–30 mM), turbidity decreased with decreasing gellan to gelatin ratio. At low calcium ion levels (2–4 mM), increasing the amount of gelatin in the mixture had a tendency to increase gel turbidity, possibly the result of light scattering from microscopically phase separated material due to incompatibility between the protein and polysaccharide (Clark, 1987). In the gels without added calcium, the high turbidity of the mixed gels may have been the result of a gellan-gelatin coacervate (Chilvers & Morris, 1987) where maximum turbidity was obtained in the range between 0.6% gellan–1.0% gelatin and 0.8% gellan–0.8% gelatin. The addition of only a small amount of calcium may have been sufficient to encourage the formation of a gellan continuous phase at temperatures higher than the gelling temperature of gelatin (Lau, Tang & Paulson, 2000) thus preventing coacervate formation.

The turbidity data did not reveal whether turbidity was caused by either the formation of more or larger

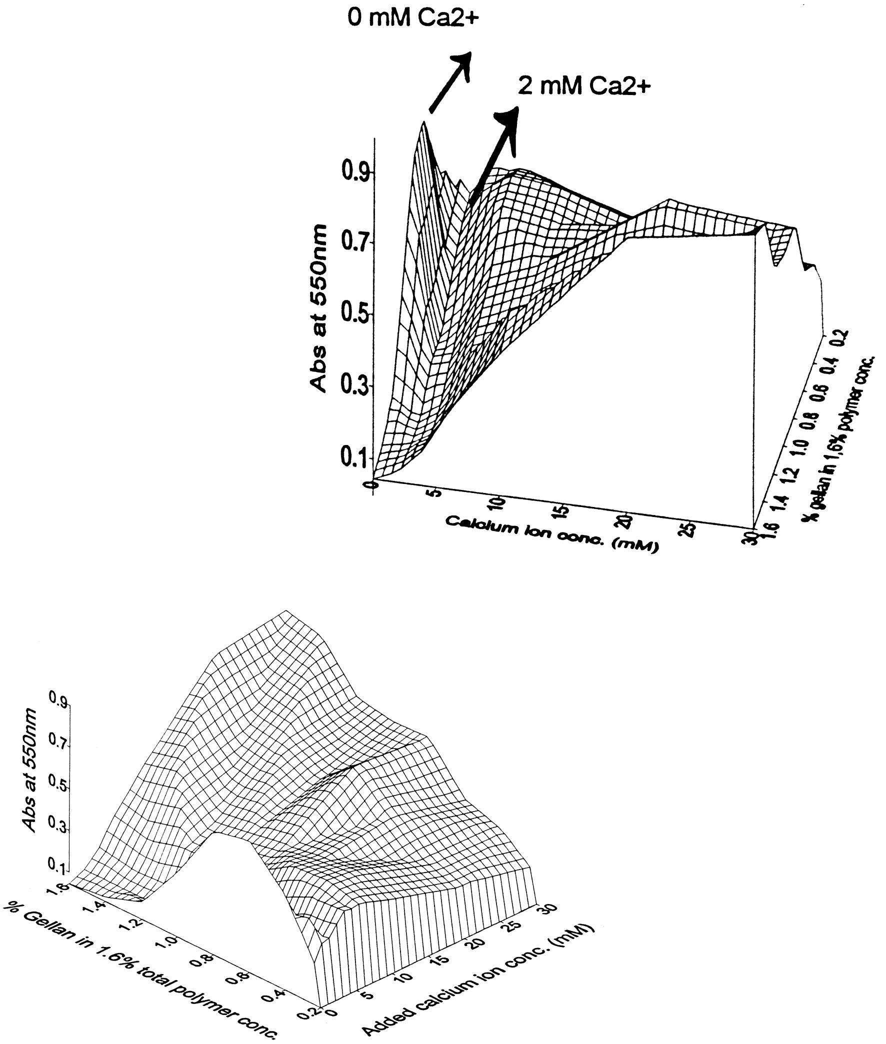

Fig. 6. Turbidity of gellan/gelatin mixed gels as a function of gellan to gelatin ratio and added calcium ion level. The 1.6% total polymer solutions contained gellan (1.6–0.2% w/v) and gelatin (0.0–1.4% w/v). The axes have been arranged to best show the treatment effects on turbidity.

aggregates, or both. However, in most cases, gel turbidity is caused by the scattering of light by particles entrapped inside the gel matrix (Kitabatake, Doi & Kinekawa, 1994). Kasapis, Morris, Norton and Clark (1993) indicated that turbidity of gelatin/maltodextrin mixed gels may be caused by intermolecular association involving extensive helix–helix aggregation. Based on the optical properties of β-lactoglobulin gels, Paulsson, Dejmek and Vliet (1990) stated that transparent protein gels consisted of a molecularly homogeneous network, whereas nontransparent gels consisted of colloid particles or aggregates (larger than one quarter of the wavelength of light above 150 nm). A study of myosin aggregates using phase contrast microscopy revealed that increased turbidity of heated fish myosin solutions was caused by the formation of more and larger aggregates (Gill, Chan, Phonchareon & Paulson 1992). Thus, microscopy should prove to be a valuable tool in elucidating the mechanisms of turbidity development in gellan/gelatin gels.

The textural and turbidity data suggest that there were two types of gel networks formed depending on ionic strength; (i) a weak interacting gellan/gelatin network at very low ionic strength, and (ii) a continuous gellan and discontinuous gelatin network when calcium ions were added. When calcium ions were added to the gellan/gelatin mixed gels, a decrease in gellan to gelatin ratio resulted in a reduction of gel hardness, which suggests a weakening of a gellan continuous phase without a compensating increase in hardness from a continuous gelatin phase or a gellan-gelatin complex. This agrees with the findings of Papagiourgiou et al. (1994) who suggested that the temperature course of gel formation and melting supported the concept of a continuous gellan phase with a discontinuous gelatin filler, even at polymer concentrations of 0.075% gellan–5% gelatin. Similarly, Fernandes, Goncalves and Doublier (1994), working with κ-carrageenan/galactomannan mixed gels, found that when sufficient KCl was added, the κ-carrageenan was capable of forming a self-supporting network which entrapped the galactomannan, and the physical properties of the mixed gels were similar to those of individual κ-carrageenan gels. In addition, Rodriguez-Hernandez and Tecante (1999) concluded that in gellan–xanthan and gellan–ι–carrageenan mixed gels, there was no interaction between the separate components and no enhancement of rheological properties.

In contrast, the hardness of the mixed gels without added calcium initially increased and then decreased gradually as the ratio of gellan to gelatin increased, suggesting a positive interaction between gellan and gelatin polymers. Morris (1990) noted that the optimum interaction of gellan gum with gelatin occurred when about equal parts of gellan and gelatin were mixed under a slightly acidic condition. Since the pH values of the gellan/gelatin mixed gels (Fig. 7) were above the isoelectric point of type B gelatin (pH 4.8–5.0; Johnston-Banks, 1990), the net charge on the gelatin molecules was negative. However, this would not preclude electrostatic interactions between positively charged regions of the gelatin molecules with anionic gellan molecules since at intermediate to high levels of gelatin, the pH of the solutions would be quite close to the isoelectric point. This is consistent with the greatest hardness and turbidity of gellan/gelatin gels without ions occurring at approximately equal ratios of the two polymers since gelatin in greater proportion would reduce the amount of gellan available for complex formation. With the addition of calcium ions, the electrostatic interactions would be diminished at the same time as gellan helix association and aggregation would be favoured, thus promoting a gellan continuous phase.

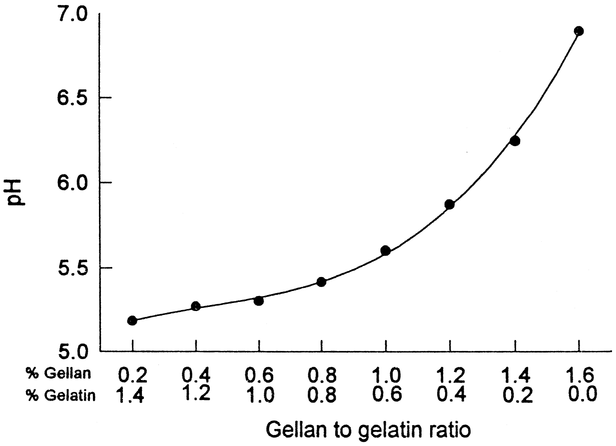

Fig. 7. pH of gellan/gelatin mixed gels without the addition of calcium ions as a function of gellan to gelatin ratio.

4. Summary

The textural properties and turbidity of gellan/gelatin mixed gels were strongly affected by the calcium ion concentration as well as the gellan to gelatin ratio ($P < 0.05$). This study demonstrated that alteration of the relative gellan to gelatin ratio at different calcium ion levels can provide a wide range of textural parameters and turbidity for gel formulations in different foods. For example, formulation of gels for a fat replacer application might be achieved by using 0.6% gellan–1.0% gelatin with no added calcium ions, as this combination has fat-like softness and opacity. Gel hardness can be increased by the addition of suitable amounts of calcium ions to the polymer solutions and increasing the ratio of gellan to gelatin, whereas brittleness, springiness and cohesiveness were very sensitive to low levels of added calcium (0–10 mM), but less sensitive to higher calcium concentrations and gellan/gelatin ratio. In general, the addition of calcium ions caused gels to be more brittle and less cohesive and springy. Turbidity was a complex function of gellan/gelatin ratio and calcium ion concentration. The least turbid gels were those which contained no gelatin and low levels of calcium, whereas maximum turbidity without the addition of calcium was observed in gels with approximately equal amounts of gellan and gelatin. The results obtained from this study suggested a weak positive interaction between gellan and gelatin when no calcium was added, whereas at higher calcium levels gellan formed a continuous network and gelatin a discontinuous phase.

Acknowledgements

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