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Effect of calcium concentration on textural properties of high and low acyl mixed gellan gels

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Abstract

Textural properties of 1% low and high acyl gellan gels and their mixtures were studied using compression tests and the microcentrifuge-microfiltration based water holding capacity (WHC) method. Low acyl (1% LA), high acyl (1% HA) gels and mixtures of 1% 25/75 LA/HA, 50/50 LA/HA, 75/25 LA/HA gels with calcium concentrations ranging from 2 to 80 mM were studied. HA or mixed gels with a lower LA/HA ratio had a greater WHC and failure strain than that of LA or mixed gels with a higher LA/HA ratio. Gellan gels with a higher LA/HA ratio had a larger initial Young's modulus. Our study also indicates that a higher LA/HA ratio does not necessarily result in a gel with a larger failure stress, although LA gels are generally firmer than HA gels. Gel strength and WHC of HA and LA/HA mixtures may reflect both stabilization and destabilization effects of glycerate groups positioned at one of the glucose residues in each repeating tetrasaccharide unit of HA gels.

Keywords: Gellan; Low acyl; High acyl; Mixed gel; Water holding capacity; Strain; Stress; Modulus

1. Introduction

Gellan gum is a linear anionic polysaccharide secreted by the bacterium *Sphingomonas elodea*. Gellan polymers consist of tetrasaccharide (ABCD) repeating units, (→ 3)-β-D-Glcp-(1 → 4)-β-D-GlcpA-(1 → 4)-β-D-Glcp-(1 → 4)-α-L-Rhap-(1 →) (Jansson, Lindberg, & Sandford, 1983). In native gellan, also called high acyl (HA) gellan, glucose residue-A contains a substitution of a L-glycerate group at C_2 and an acetate group at an approximate 50% replacement level at C_6 (Kuo & Mort, 1986). Commercial low acyl (LA) gellan gum is produced by removing the acetate and glycerate groups with a strong alkali treatment.

Both low and high acyl gellan molecules form a three-fold double-helical structure under an appropriate aqueous environment (Chandrasekaran, Millane, & Arnott, 1988). The aggregation of double-helical segments leads to a three-dimensional network. In high acyl gellan polymer, the acetate group positioning on the periphery of the double helix exerts no steric hindrance on an isolated gellan double helix and may have little or no effect on the aggregation of gellan double helices (Chandrasekaran & Thailambal, 1990). The glycerate group locating in the interior of the double helix forces the carboxylate group in the glucuronate residue-B to rotate, changing the ion binding characteristics to ease steric hindrance. This may explain the weak gelation properties of high acyl gellan (Chandrasekaran, Radha, & Thailambal, 1992; Chandrasekaran & Thailambal, 1990).

The glycerate group in glucose residue-A of high acyl gellan also has stabilizing effects on the double helix structure. The three oxygen atoms in the glycerate group specifically stabilize the double-helical structure by forming four new inter- or intrachain hydrogen bonds and participating in ion binding (Chandrasekaran & Thailambal, 1990). The substitution of the glycerate group and the resultant stereochemical changes leads to the loss of several inter- or intrachain hydrogen bonds; however, a net increase of four hydrogen bonds (from 6 to 10) was identified for HA gellan compared to LA gellan polymers (Chandrasekaran et al., 1988; Chandrasekaran et al., 1992; Chandrasekaran & Thailambal, 1990). Although high acyl gellan gels are normally softer than low acyl gellan gels, the structure of high acyl gellan gels are intrinsically more stable. One attribute supporting this premise is the much higher temperature for conformational ordering of high acyl gellan

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compared to low acyl gellan (Morris, Gothard, Hember, Manning, & Robinson, 1996).

Cations stabilize the gellan three-dimensional network by direct cross-linking with divalent cations, such as Ca^{++}, or indirect cross-linking with monovalent cations, such as K^+ (double helix—K^+—water—K^+—double helix), two double helices through carboxylate groups in the glucuronate residue-B (Chandrasekaran & Thailambal, 1990).

The rheological properties, such as yield stress and gelling temperature, as well as physical properties, such as water holding capacity (WHC), reflect the stabilization and destabilization effects of the glycerate group in the formation of three-dimensional structures for high acyl gellan compared to low acyl gellan.

Gel structures retain a large amount of water. The ability of gels to retain the water is an important parameter for characterization of the gels. WHC is generally expressed as the amount of water the gel structure can hold, or the capability of the gel to retain this water during storage or when subjected to external forces. WHC of the gel is determined by the gelation mechanism. WHC is an important quality parameter for food gels since WHC affects textural properties and product costs.

The objective of this study was to investigate the effect of calcium concentration and LA/HA ratio on the WHC and rheological properties, including failure strain, failure stress and initial Young's modulus, of LA and HA gellan gels and gel mixtures prepared from various ratios of LA and HA components.

2. Materials and methods

2.1. Gel preparation

Both low acyl (Kelcogel F) and high acyl (Kelcogel LT100) gellan gum were provided by CP Kelco US, Inc. (Wilmington, DE). LA, HA, and mixtures of 25/75 LA/HA, 50/50 LA/HA and 75/25 LA/HA were used for the study. One percent hot gellan solutions with calcium concentrations ranging from 2 to 80 mM (2, 4, 6, 8, 15, 30, 40, 60, 80 mM) were prepared as previously described (Huang, Tang, Swanson, Cavinato, Lin, & Rasco, 2003). The hot gellan solutions were poured into preheated (98 °C) stainless steel tubes (inner diameter 21 mm, length 21 cm) for compression tests or into polypropylene tubes (inner diameter 6.3 mm, length 7.5 cm) for WHC tests. All gels were maintained at room temperature (ca. 22 °C) for 24–28 h until further analyzed.

2.2. Water holding capacity test

Gel was removed from polypropylene tubes and a 15 mm long section was removed from the center of each gel and placed into a centrifugal filter unit (Ultrifree-MC; Millipore Corp., Bedford, MA). Gels were centrifuged (Centrifuge 5414C; Brinkman Instruments, Inc., Westbury, NY) at room temperature at 2000 rpm (224*g*) for scheduled time intervals (1, 3, 5, 10, 15, 20, 30, 60 min). The distance between the center of rotor and the center of sample was 5.0 cm. Gels were weighed immediately after each time interval of centrifugation. A relative small centrifugation force was applied to better understand how centrifugation time affected the WHC of gels (Kocher & Foegeding, 1993; Mao, Tang, & Swanson, 2001). In this study, WHC was defined as the ratio (%) of gel weight after centrifugation to the original gel weight and was determined in duplicate.

2.3. Compression test

Gels removed from the metal tube were cut into 21 mm length pieces. The cylindrical gel sections were then placed between a flat aluminum probe (diameter 50 mm) and flat aluminum surface of a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Codalming, Surrey, UK). The gel was compressed by the flat aluminum probe at a cross-head speed of 0.3 mm/s until failure (reaching maximum engineering stress) (Mao, Tang, & Swanson, 2000; Tang, Tung, & Zeng, 1995). Compression tests were repeated five times for each gel. Hencky's normal strain (ε_h) and corrected stress (σ_h) were calculated from the engineering strain (ε_{eng} = reduction of length/original length) and engineering stress (σ_{eng} = force/original cross-section area) to take into account the enlarged cross-sectional area of the gels as they are compressed (Tang, Tung, & Zeng, 1996) as follows:

$$\varepsilon_h = -\ln(1 - \varepsilon_{eng}) = \ln(\text{original length/length after compression}) \quad (1)$$

$$\sigma_h = \sigma_{eng}(1 - \varepsilon_{eng}) = \sigma_{eng}(\text{length after compression/original length}) \quad (2)$$

The failure strain and failure stress were determined from a compression curve (Mao et al., 2000; Tang et al., 1995).

The initial Young's modulus, an indicator of gel firmness, was determined using the procedure of Mao et al. (2000) with some minor changes. In this study, the initial Young's modulus was derived from the slope of the regression line of the engineering stress vs. engineering strain for strain up to 5%. Unlike Mao et al. (2000), engineering stress and engineering strain were used for the initial Young's modulus derivation because the change of the cross-sectional area was minor and negligible for a compression below 5% strain.

Since high acyl gellan gels were very soft and deformed under gravity, no compression tests were conducted for the pure high acyl gellan gels in this study.

3. Results and discussion

3.1. Water holding capacity

Low acyl gellan gels rapidly lost water when subjected to centrifugal force of 224*g* (Fig. 1(a) and (b)). The calcium concentrations in LA gels greatly affected WHC. The difference in WHC among gels with selected concentrations of added Ca^{++} were more than 60% (e.g. between 2 and 40 mM gels after 10 min centrifugation). For a LA gel with less than 40 mM added Ca^{++}, WHC of the gel decreased with increase of Ca^{++}; however, once the added Ca^{++} was more than 40 mM, WHC of a LA gel increased with further addition of Ca^{++}.

High acyl gellan gels exhibited a greater WHC than LA gels (Fig. 2). All treatments of HA gels exhibited more than 84% WHC after 60 min centrifugation. In addition, HA gels were less sensitive to changes in Ca^{++} concentration, which was similar to the results from a previous study on gellan gels with near infrared methods (Huang et al., 2003). The largest difference among high acyl gels with different Ca^{++} treatments was less than 5% during the 60 min centrifugation.

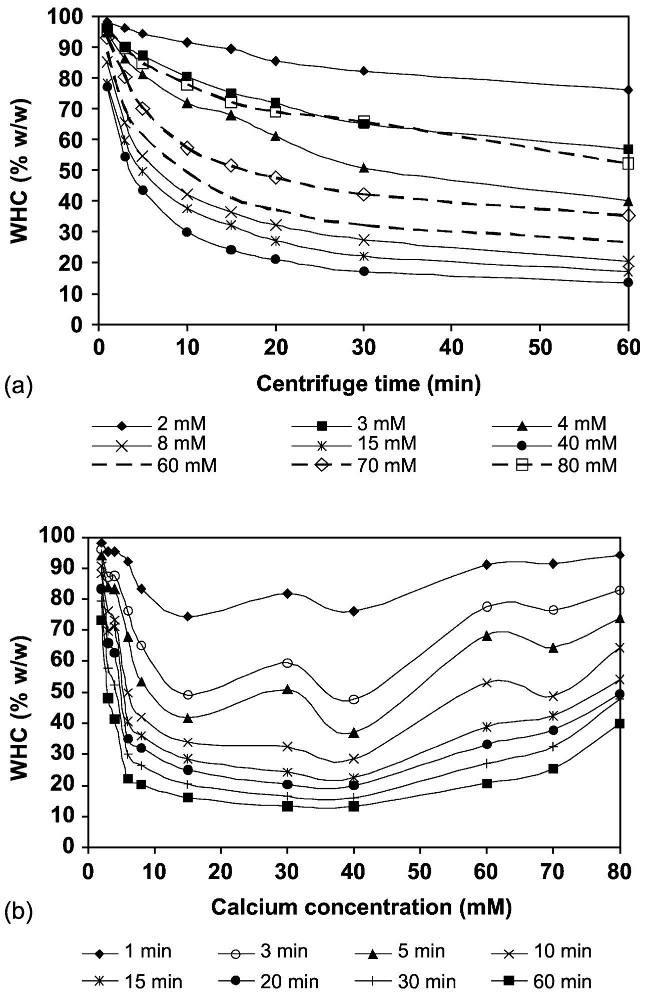

Fig. 1. Change in of WHC of LA gellan gels with (a) centrifuge time, and (b) calcium concentration when subjected to 224*g* centrifugal force at ca. 22 °C.

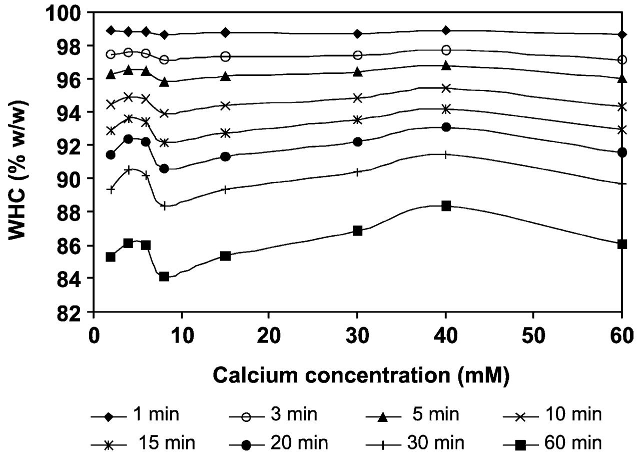

Fig. 2. Water holding capacity of high acyl gellan gels when subjected to 224*g* centrifugal force at ca. 22 °C.

High acyl gellan gels exhibited better WHC than LA gels, which may be because HA gels contain more hydrogen bonds than LA gels in the double helical backbone (Chandrasekaran et al., 1988; Chandrasekaran et al., 1992; Chandrasekaran & Thailambal, 1990). Compared to the LA gels, the glycerate group in HA gels changes the ion binding characteristics of double helical backbone and thus affects the sensitivity to calcium concentration (Chandrasekaran et al., 1992; Chandrasekaran & Thailambal, 1990). This may explain why change in calcium concentration resulted in a much smaller effect on the WHC of HA gels compared to LA gels.

WHC of mixtures of LA and HA were between that of LA and HA gels (Fig. 3). The effects of calcium concentration on the WHC of mixed gels followed a similar

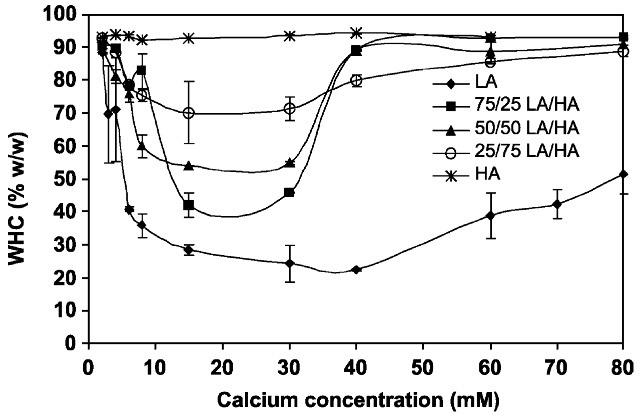

Fig. 3. Water holding capacity of gellan gel mixtures with selected calcium concentrations after 15 min centrifugation (224*g*, ca. 22 °C).

pattern. Mixed gels exhibited similar WHC as their HA gel counterparts at low Ca^{++} (2 mM) and high Ca^{++} concentrations (80 mM). In addition, the ratio of LA/HA in the mixed gels clearly affected the WHC of the gels with calcium concentrations ranging from 8 to 30 mM, and mixed gels with a larger LA content have a lower WHC.

3.2. Rheological properties

3.2.1. Failure strain

The failure strain of LA/HA mixed gels (Fig. 4) resulted in trends similar to the previous report by Mao et al. (2000). That is, the smaller the LA/HA ratio, the more flexible (larger failure strain) the gel. However, Mao et al. (2000) reported that the failure strain of a mixed gel was significantly influenced by the addition of a small amount of calcium (2–14 mM). This influence on failure strain disappeared when calcium concentration reached 20 mM as the failure strain reached a constant value. In our study, we also observed a significant effect on failure strain with a small amount of calcium, but there was no constant values obtained with further increase of calcium concentration.

The discrepancy between results from these studies can be explained by the use of different gel preparation procedures that could have created gels with different physical properties. In our study, gel solutions were cooled to ambient temperature by holding the gel dispersions at room temperature. In the Mao et al. (2000), gel solutions were cooled rapidly by immersing the gel molds in 15 °C running water for 15 min. Cooling rate of a gel solution affects the gelation temperature, gel clarity and texture properties of gellan gels (Tang, Mao, Tung, & Swanson, 2001; Tang, Tung, & Zeng, 1997;). The cooling rate may affect the size of the junction zones of gels and thus change the textural properties of gels (Tang et al., 2001).

Increases in calcium concentration (Fig. 4) resulted in an increase in the failure strain of 1% LA gels from ca. 0.32 (< = 6 mM Ca^{++}) to a maximum value of 0.96 (30 mM Ca^{++}), and then a decrease to ca. 0.30 for Ca^{++} concentration from 30 to 80 mM. The variation (0.66) of the failure strain of LA gels with the change of calcium concentrations was much larger than that observed for mixed gels, which may be another indication that low acyl gels are more sensitive to the change in calcium concentration.

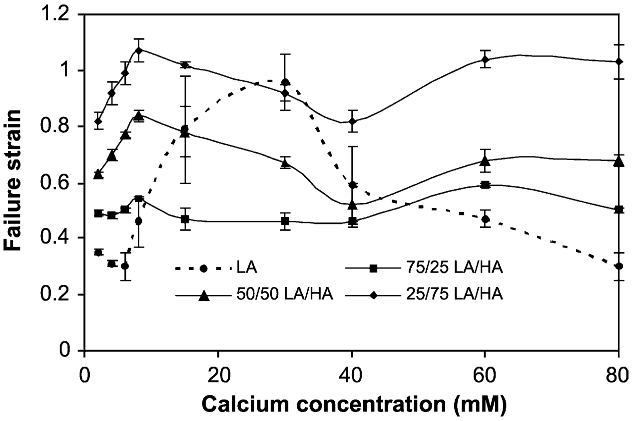

Fig. 4. The failure strain of low acyl and mixed gellan gels with selected calcium concentrations.

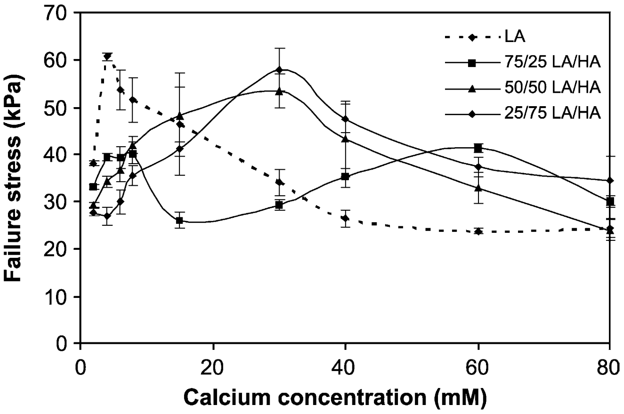

Fig. 5. The failure stress of low acyl and mixed gellan gels with selected calcium concentrations.

3.2.2. Failure stress

Failure stress is an indication of gel strength. Fig. 5 presents the failure stress of 1% LA and mixed gels with selected Ca^{++} concentrations. The failure stress reached a peak at 30 mM for 50/50 LA/HA and 25/75 LA/HA mixed gels, and at 4 mM for LA gels. However, the failure stress of the 75/25 LA/HA mixed gels exhibited a different pattern from that of 50/50 LA/HA and 25/75 LA/HA gels. Instead of one peak, the 75/25 LA/HA mixed gels had two peaks, at 8 and 60 mM Ca^{++} concentration. Two peaks observed for 75/25 LA/HA mixed gels may reflect two different gelation mechanisms for LA and HA gels, respectively. Mixed gels with more than 50% HA followed a pattern similar to that of HA gels.

Although LA gellan generally forms firmer gels than HA gellan, mixed gels with higher LA/HA ratio may not exhibit greater failure stress. Our study demonstrated that the range of the failure stress for LA and three different mixed gels with selected calcium concentrations were within similar range (24–60 kPa). The failure stress represents the maximum external force that a gel can tolerate without fracturing. HA gellan forms soft but very elastic gels and LA gellan forms firm but brittle gels. Mixed gels with higher HA/LA ratio exhibited higher failure strains, indicating the gel did not break or fracture more easily. This implies the possibility that a soft gel may have relative high failure stress.

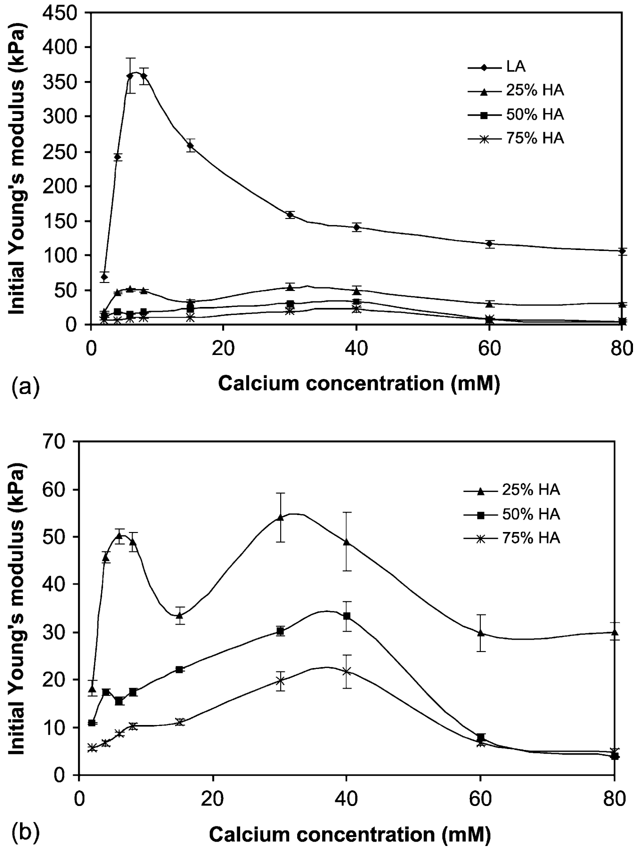

Fig. 6. (a) The initial Young's modulus of low acyl and mixed gellan gels with selected calcium concentrations. (b) The initial Young's modulus of LA/HA mixed gels with selected calcium concentrations.

3.2.3. Initial Young's modulus

Initial Young's modulus for 1% LA gels were much larger than those for 1% LA/HA mixed gels. Also, mixed gels with a higher LA/HA ratio exhibited larger initial Young's modulus than gels with a lower LA/HA ratio at equivalent calcium concentrations (Fig. 6). Since LA formed firmer gels than HA, and initial Young's modulus is an indication of gel firmness, a mixed gel with a larger LA/HA ratio was generally expected to exhibit a larger initial Young's modulus. The effect of LA/HA ratio on the initial Young's modulus for the mixed LA/HA gels was opposite to that of failure strain, for which the larger the LA/HA ratio, the smaller the failure strain.

changing calcium concentration yielded similar effects on the initial Young's modulus as on failure stress. For low acyl gels with increase in calcium concentrations, the initial Young's modulus increased rapidly and reached a peak at 6–8 mM Ca^{++} concentration, and then decreased to a constant value. The failure stress for LA gels as a function of calcium concentration followed a similar trend as the initial Young's modulus, although the peak was around 4 mM for the failure stress instead of 6–8 mM for the initial Young's modulus. The initial Young's modulus reached a peak at around 40 mM for 50/50 LA/HA and 25/75 LA/HA mixed gels, which was similar to the trend observed in failure stress reaching a peak at around 30 mM. The 75/25 LA/HA mixed gels also exhibited two peaks (at 6 and 30 mM Ca^{++} concentrations) instead of a single peak. This may be related to the initial Young's modulus peak of LA gels at 6–8 mM, and possibly to HA gel at around 40 mM Ca^{++} concentration.

4. Conclusions

High acyl gellan gels or mixed gels with a smaller LA/HA ratio exhibited larger water holding capacities and larger failure strains than low acyl gellan gels or mixed gels with larger LA/HA ratios. These features may result from the stabilization effect of glycerate groups on the three-dimensional structure of HA gels creating inherently more stable gels. Gellan gels with a larger LA/HA ratio exhibited a larger initial Young's modulus and this may be related to the special role glycerate groups play in destabilizing the three-dimensional structure. Our results also indicated that a larger LA/HA ratio does not necessarily result in gel with a larger failure stress, although gels containing greater quantities of LA were generally firmer than gels containing larger quantities of HA.

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