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Original article

Influence of temperature, calcium and sucrose concentration on viscoelastic properties of *Prosopis chilensis* seed gum and nopal mucilage dispersions

Gipsy Tabilo-Munizaga,[1]* Carmen Sáenz-Hernández[2] & Carolina Herrera-Lavados[1]

1 Food Engineering Department, Universidad del Bío-Bío, Av. Andrés Bello 720, Chillán, CP 3780000, Chile
2 Agricultural Industry and Enology Department, Universidad de Chile, Av. Santa Rosa 11315, Santiago, CP 8820808, Chile

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Summary Viscoelastic properties of two nontraditional hydrocolloid dispersions were evaluated. *Prosopis chilensis* seed gum was evaluated based on temperature (5–80 °C) and added $CaCl_2$ (0.07%), whereas nopal mucilage was evaluated based on temperature (5–80 °C) and sucrose concentration (0–20%). Viscoelasticity was tested by the small strain oscillatory shear test; storage modulus (G′), loss modulus (G″) and tan δ were reported. *Prosopis chilensis* and nopal dispersions behaved as weak gels (G' > G'') regardless of experimental condition. Raising temperature from 20 to 80 °C significantly increased G'. The gel structure was strengthened by adding $CaCl_2$ and G' increased at 40 °C. The sucrose effect depended on concentration and temperature; at low sucrose concentrations, G' modulus increased regardless of temperature level, but at high concentrations, it decreased at temperatures >40 °C. In conclusion, nopal and *Prosopis chilensis* dispersions show weak gel structure regardless of experimental condition. G′ increases as temperature increases, and these dispersions could be suitable for food applications requiring heat tolerance.

Keywords Dispersions, hydrocolloids, nopal mucilage, *Prosopis chilensis* seed gum, viscoelastic properties.

Introduction

Hydrocolloids are high molecular weight biopolymers, which are excellent thickening and stabilising agents. Their absence of toxicity allows their use in food and pharmaceutical industries, improving the long-term stability of food systems (Estévez *et al*., 2004). The presence of many hydroxyl groups on the hydrocolloid structure increases their affinity to bind water and makes them highly soluble in this solvent to produce a dispersion, which is an intermediate between a true solution and a suspension (Li & Nie, 2016). The rheological properties of hydrocolloids are particularly important when used in food formulations because of their effects on textural attributes and as stabilising, thickening, gelling and emulsifying agents (Coelho *et al*., 2017). While all hydrocolloids have a thickening effect, some of them are also able to form gels. Gels consist of polymer molecules that are cross-linked to form tangled and interconnected molecular networks immersed in a liquid medium, and this medium is water in food systems (Saha & Bhattacharya, 2010). Because gels are viscoelastic materials, dynamic rheological tests evaluate gel system properties that are well suited for studying different gum and hydrocolloid characteristics (Rincón *et al*., 2014; Busch *et al*., 2015). In the dynamic rheological test, a strain within the viscoelastic region is continuously applied on a tested sample to define its elastic or viscous behaviour to determine whether the material is solid or liquid-like (Tabilo-Munizaga & Barbosa-Cánovas, 2005). Such studies about hydrocolloid rheology help to better characterise their structure, which plays an important role in process design and quality control, and also provide data for mixing, pumping, packing operations and compatibility with manufacturing equipment (Rincón *et al*., 2009).

The most used hydrocolloids are starch, xanthan gum, guar gum, locust bean gum, gum karaya, gum tragacanth, gum Arabic, alginate, pectin, carrageenan, gelatin, gellan and agar (Saha & Bhattacharya, 2010). Plant hydrocolloids have an advantage over those obtained from animals because of their consumer-friendly image, and this has led to the search for new nontraditional sources. *Prosopis* seed gum is obtained from the seeds of the mesquite tree, locally known as 'Algarrobo' (*Prosopis chilensis* (Mol) Stuntz). This

*Correspondent: E-mail: gtabilo@ubiobio.cl

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hydrocolloid possesses high water-binding properties and generates viscous solutions even at low concentrations (Matsuhiro *et al.*, 2006; Estévez *et al.*, 2012). On the other hand, nopal mucilage is a high molecular weight hetero-polysaccharide obtained from *Opuntia ficus-indica* plants; it is used in the cosmetic, medicinal and food industries (Contreras-Padilla *et al.*, 2016). Nopal is considered as a functional food with many nutraceutical properties, including high dietary fibre and antioxidant content (Angulo-Bejarano *et al.*, 2014). The viscoelastic properties of nopal mucilage from fresh and dried cladodes have been studied (Contreras-Padilla *et al.*, 2016), but the effect of different temperatures and sweeteners on its viscoelastic behaviour has not been reported. Even if *Prosopis* chilensis seed gum has been gaining attention as a novel hydrocolloid, there is a current lack of information about its viscoelastic behaviour (Astudillo *et al.*, 2000; Estévez *et al.*, 2004; Schmeda-Hirschmann *et al.*, 2015). As the purpose of hydrocolloids is food or pharmaceutical applications, it is important to determine the effect of different conditions that can be found during a production process, such as temperature changes, ion presence and sweeteners. Hydrocolloid characteristics can also be modified by including additives, thus obtaining new uses for the same gum depending on the applied process. Therefore, the aim of this work was to determine the viscoelastic properties of *Prosopis chilensis* seed gum and nopal mucilage dispersions as affected by temperature and adding $CaCl_2$/sucrose.

Materials and methods

Raw materials

Prosopis chilensis seed gum and nopal mucilage were obtained by acid extraction and supplied by the Agroindustry and Enology Department at the Universidad de Chile, Chile. The extraction was performed with 72% w/v sulphuric acid at 80 °C for 20 min. The wet gums were dried at 37 °C for 16 h and then milled to 60 mesh to obtain a fine powder (Estévez *et al.*, 2004). All reagents were of analytical grade.

Dispersion formulation

The particle size was standardised to 60–75 microns for *Prosopis chilensis* seed gum and 50–60 microns for nopal mucilage using sieves of 250, 150, 100, 75, 63 and 50 microns. Dry *Prosopis chilensis* seed gum was hydrated at room temperature for 16 h with distilled water at concentrations of 0.4% and 0.6% w/v. Dispersions were agitated in a magnetic stirrer for 90 min at 50 °C, and $CaCl_2$ (70 mg per 100 mL) was added as needed. As for nopal mucilage, dry powder was dispersed in distilled water to achieve a concentration of 0.5% w/v. Dispersions were mixed at room temperature for 120 min, and sucrose was added at concentrations of 0%, 10%, 15% and 20% (w/v). Dispersion pH was adjusted to 4.0 with 1 N HCl for *Prosopis chilensis* seed gum and 1 M citric acid for nopal mucilage. Finally, dispersions were sonicated (Transsonic 570, Elma D-78224) to eliminate air and samples stored at 4 °C until further analysis.

Viscoelastic characterisation

Once prepared, all samples were immediately rheologically measured with a rotational Physica® MCR300 rheometer (Physica® Inc., Spring, TX, USA) equipped with a cone-plate geometry (CP50-1). The sample temperature under experimental conditions (5, 20, 40, 50 and 80 °C) was controlled by a Peltier plate system attached to a water circulation unit. Oscillatory amplitude sweep test was carried out with a torque of 1–50 mN m^{-2} at 10 Hz frequency to determine the linear viscoelastic region. Frequency sweeps were then carried out with constant shear stress between 0.2% and 0.5% and frequency between 0.1 and 100 s^{-1}. The viscoelastic parameters, such as the storage modulus G' (elastic modulus), loss modulus G'' (viscous modulus) and tan δ (loss tangent), were calculated using the manufacturer's software (US200 Physica® version 2.01, USA).

Statistical analysis

The experiment was replicated three times. Each replicate considered seven samples for rheological parameters. Statistical analyses were performed using mean, standard deviation and analysis of variance (ANOVA) with Tukey's test (5% significance). Data analysis was performed with the OriginPro 2015 software (OriginLab Corporation, Northampton, MA, USA).

Results and discussion

Hydrocolloid viscoelastic behaviour

Oscillatory tests are the most used rheological methods to study the viscoelastic behaviour of foods due to their high sensitivity to chemical and physical structures. The dynamic frequency sweep test can be used to characterise a dispersion, resulting in the most common classification, which differentiates between a dilute solution, an entanglement network system (concentrated solution) and a weak or strong gel (Hesarinejad *et al.*, 2014).

Figure 1 shows the viscoelastic parameters of *Prosopis chilensis* seed gum and nopal mucilage dispersions at 20 °C. These dispersions behaved as gels in which storage modulus G' was higher than loss modulus G''

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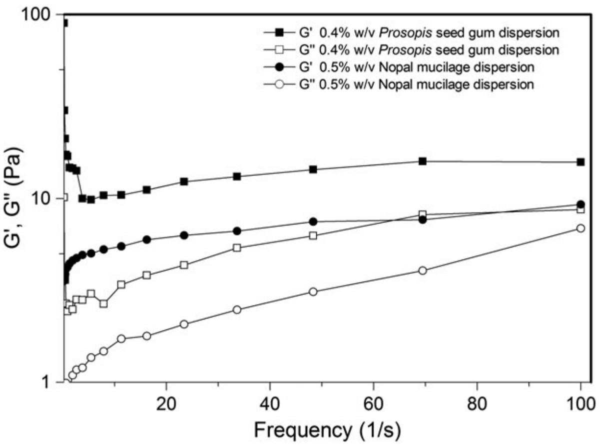

Figure 1 Mechanical spectra of *Prosopis chilensis* seed gum and nopal mucilage dispersions at 20 °C: (■) 0.5% w/v Nopal mucilage dispersion, (●) 0.4% w/v *Prosopis chilensis* seed gum dispersion (Storage modulus (G'): full symbols; Loss modulus (G''): open symbols).

in all cases; no crossover point occurred, which demonstrated that the elastic component was more important than the viscous component and that these dispersions possess a weak gel structure. Similar results have been shown by *Sterculia apetala* gum exudate dispersions (Pérez-Mosqueda *et al.*, 2013), basil seed gum (Rafe & Razavi, 2013) and *Alyssum homolocarpum* seed (Anvari *et al.*, 2016), which also behave as weak gels. However, previous studies on *Prosopis* seed gum dispersions from *Prosopis flexuosa* (2.6% and 4.2% w/w), *Prosopis juliflora* (0.6–1.4% w/v) *and Prosopis ruscifolia* (0.3% w/v) seed gum were reported to behave as entangled macromolecules rather than gels because their G'' modulus is higher than G' up to a crossover point (Ibañez & Ferrero, 2003; Rincón *et al.*, 2014; Busch *et al.*, 2015). In the case of nopal mucilage, León-Martínez *et al.* (2011) reported changes in the viscoelastic properties of nopal mucilage dispersions that depended on the drying method; they observed a predominant viscous behaviour in spray-dried reconstituted mucilage solutions at concentrations between 1% and 6% w/v mucilage. However, freeze-dried mucilage dispersions showed a gel-like behaviour at concentrations >3% w/v. According to Medina-Torres *et al.* (2000), nopal mucilage dispersions behave as viscoelastic solutions at concentrations below 5% w/v mucilage, but they turn into soft gels as the hydrocolloid concentration increases up to 10% w/v, thus indicating a tendency to form macromolecular networks with a more important elastic component. Differences between previously reported *Prosopis* and nopal mucilage dispersions, and our results could be explained by the dissimilarities in the purification and isolation method used and because none of the previous studies have isolated *Prosopis* seed gum or nopal mucilage by acid extraction. Two frequently used methods to extract hydrocolloids are acid or alkaline extraction, and each method leads to different gum characteristics. Previous studies in *Prosopis* seed gum by Estévez *et al.* (2004) reported that gums obtained by acid extraction had reduced protein content and increased dispersion viscosity compared with gums obtained by alkaline extraction; this effect is caused by the formation of new hydrogen bonds by acid extraction instead of the reduction in molecular weight and suppression of intermolecular associations caused by alkaline extraction (Goycoolea *et al.*, 1995). The increase in hydrogen bond formation produced by acid extraction could also explain the high G' values obtained for nopal and *Prosopis* dispersions in this study.

Effect of temperature on hydrocolloid viscoelastic behaviour

The mechanical spectrum of nopal mucilage at different temperatures (5, 20, 40, 60 and 80 °C) is shown in Fig. 2 in which a fixed shear stress within the linear viscoelastic range was applied. While increasing the temperature from 5 to 20 °C did not significantly change G' or G'' modulus values (G'' data not shown), a significant increase in G' values was found when the temperature gradually increased from 20 to 80 °C; this indicates that molecular chains are better able to form a gel at higher temperatures. It can be noted that temperatures less than 20 °C are associated with G' values below 100 Pa, while temperatures greater than 40 °C are related to G' values above 10 000 Pa (Fig. 2). The increase in G' modulus is related to the strengthening of a three-dimensional

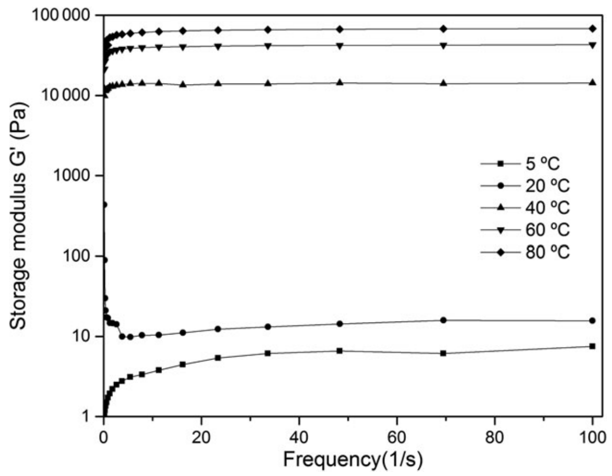

Figure 2 Effect of temperature on storage modulus (G') of 0.5% nopal mucilage dispersion.

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network in which new hydrogen bonds are forming within gum molecules. A high temperature could also enhance the thickening effect that gums exert on fluid mobility (Wei *et al.*, 2015). In contrast, previous work by Medina-Torres *et al.* (2000) on nopal mucilage viscoelasticity stated that increasing the temperature from 5 to 35 °C reduced G' modulus in dispersions with a 5% w/v mucilage concentration; however, this mucilage was obtained by alkaline extraction, and its structure differed from the acid-extracted mucilage, which could be the cause of this different behaviour.

For *Prosopis chilensis* seed gum dispersions, the effect of temperature is shown in Fig. 3a, b; two behaviours can also be identified: low G' (5 and 20 °C) values (<10 Pa) and high G' (60 and 80 °C) values (>10 000 Pa), while the behaviour of samples at 40 °C depends on gum concentration. At 0.4% gum concentration, *Prosopis chilensis* dispersion behaviour was similar to nopal dispersions; however, when gum concentration increased to 0.6%, dispersions at 40 °C showed low G' with no significant differences between 5 °C (12 Pa), 20 °C (14 Pa) and 40 °C (25 Pa). These results indicate that the increase in gum concentration retards the formation of the three-dimensional network structure induced by temperature.

The G' modulus remained higher than the G'' modulus at all temperatures (G'' is not represented in the figures); this suggests that nopal mucilage and *Prosopis*

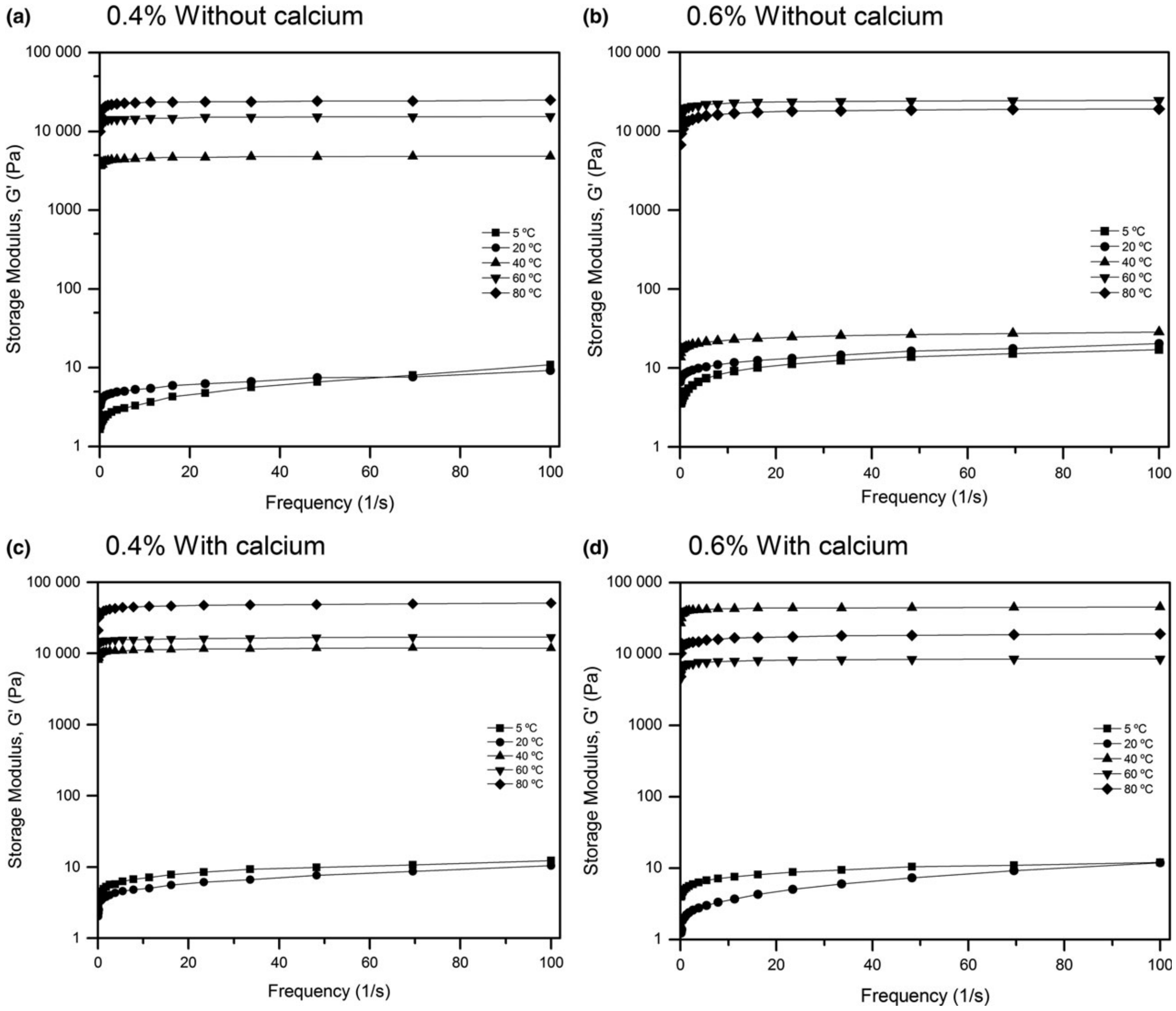

Figure 3 Effect of temperature and $CaCl_2$ on storage modulus (G') of *Prosopis chilensis* seed gum dispersion at: (a) 0.4% w/v gum, (b) 0.6% w/v gum, (c) 0.4% w/v gum with $CaCl_2$ and (d) 0.6% w/v gum with $CaCl_2$.

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chilensis seed gum dispersions remained in a weak gel state regardless of applied temperature.

Another parameter that can be used to evaluate the viscoelastic behaviour is the loss tangent (tan δ); this is the ratio between G'' loss modulus and G' storage modulus, that is, the energy lost per cycle divided by the energy stored per cycle (Steffe, 1996). When tan $\delta < 1$, elastic behaviour is predominant, while tan $\delta > 1$ indicates a predominant viscous effect. If the tan δ value is greater than 0.01, the solutions are not real gels because the structure is halfway between a concentrated solution and a real gel, so that they can be considered as weak gels (Naji-Tabasi & Razavi, 2017). Fig. 4 shows tan δ values for *Prosopis chilensis* seed gum dispersions; tan δ was less than one under all the conditions, indicating that dispersions are more elastic than viscous. Viscous modulus (G'') contributed more to viscoelastic dispersion at 5 °C (tan $\delta > 0.4$); however, elastic modulus (G') became more important as the temperature increased and tan δ decreased by 40% and 53% for 0.4% and 0.6% w/v, respectively; this means that a stronger intertwined network is formed when the temperature increases. These results are in accordance with G' values obtained for the *Prosopis chilensis* seed gum dispersions because G' modulus increased when temperatures increased and the gel matrix was strengthened. Similar results were obtained in *Lepidium perfoliatum* seed gum dispersions in which an increase in temperature from 5 to 85 °C decreased tan δ values between 32% and 69%, suggesting the presence of hydrophobic interactions strengthened by temperature (Hesarinejad *et al*., 2014).

Effect of adding $CaCl_2$ on viscoelastic behaviour of *Prosopis chilensis* seed gum

Hydrocolloids form gels through the association of their polymer chains by hydrogen bonding, hydrophobic association and cation-mediated cross-linking. In some polymers, the presence of gel-promoting cations, such as Na^+ and Ca^{+2}, promotes the formation of junction zones, which creates an interconnected three-dimensional network. As more junction zones develop, the polymer solution is transformed into a more solid state (gel) (Nickerson *et al*., 2003). The data shows that there is no clear trend based on temperature or concentration on the effect of adding $CaCl_2$ to *Prosopis chilensis* seed gum dispersion (Fig. 4); however, a notorious effect was observed at 40 °C where $CaCl_2$ significantly increased G' modulus in 240% for samples with 0.4% w/v gum and 150,000% for samples with 0.6% w/v gum concentration (Fig. 3e, d); however, this increase did not affect ($P < 0.05$) tan δ at 0.6% w/v gum concentration because G'' also increased in a similar magnitude. The highest G' values for *Prosopis chilensis* dispersions were also obtained by adding $CaCl_2$ at 80 and 40 °C for 0.4% and 0.6%, respectively. Ca^{+2} produces salt-induced gelation that can be attributed to an increase in the electrostatic interaction between the polymer chains and charged cations (Xu *et al*., 2015a). However, the effect of Ca^{+2} on hydrocolloids depends on the type of gum and total ionic concentration. Bao *et al*. (2016) reported that adding $CaCl_2$ to *Auricularia auricular-judae* dispersions reduced G' and G'' modulus, probably because the added salt screened the ionic charge of the polysaccharide causing decreased cross-linking junctions between polymer chains. A similar behaviour was observed in *Descurainia Sophia* seed gum solutions in which $CaCl_2$ decreased G' and G'' at concentrations between 10 and 100 mM (Sherahi *et al*., 2018). On the other hand, Pérez-Campos *et al*. (2012) showed that dilute gellan solutions with $CaCl_2$ changed their behaviour from macromolecular solutions to weak and true gels as the total ionic concentration of the system increased. We observed an effect that depended on temperature, $CaCl_2$ and gum concentration. At a low gum concentration, adding $CaCl_2$ improved the gelation effect induced by temperature, thus increasing G' and reaching a maximum value at 80 °C. Furthermore, when gum concentration increased, adding $CaCl_2$ strengthened the gel network at low temperatures (<40 °C) and reached its maximum G' value at 40 °C; however, a further increase in temperature destabilises the network and reduces viscoelasticity. This effect could be attributed to reduced intermolecular interactions and molecular entanglements between polymer molecules produced by the screening of its charge at this specific ionic concentration together with the increased motion of molecules and distortion of hydrogen bonds occurring at high temperatures (Xu *et al*., 2015b; Sherahi *et al*., 2018).

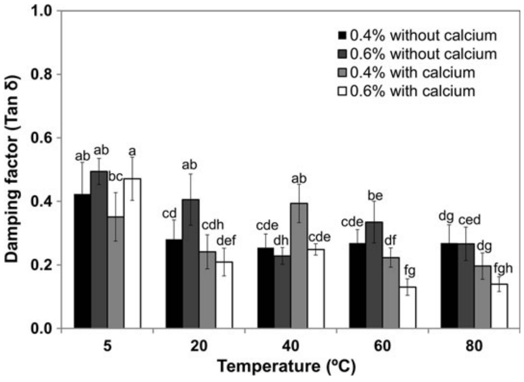

Figure 4 Effect of temperature on damping factor of *Prosopis chilensis* seed gum dispersion. Different lowercase letters (a, b, c, d, e, f, g, h) denote statistically difference between groups ($P < 0.05$).

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Effect of sucrose concentration on viscoelastic behaviour of nopal mucilage dispersion

The effect of adding sucrose not only depends on the sugar concentration but the applied temperature (Fig. 5). The addition of sugar at a low concentration (10% w/w) increased ($P > 0.05$) G' modulus with respect to the control, and this effect was observed at all assayed temperatures. A similar trend was identified with 15% sucrose between 20 and 60 °C where G'

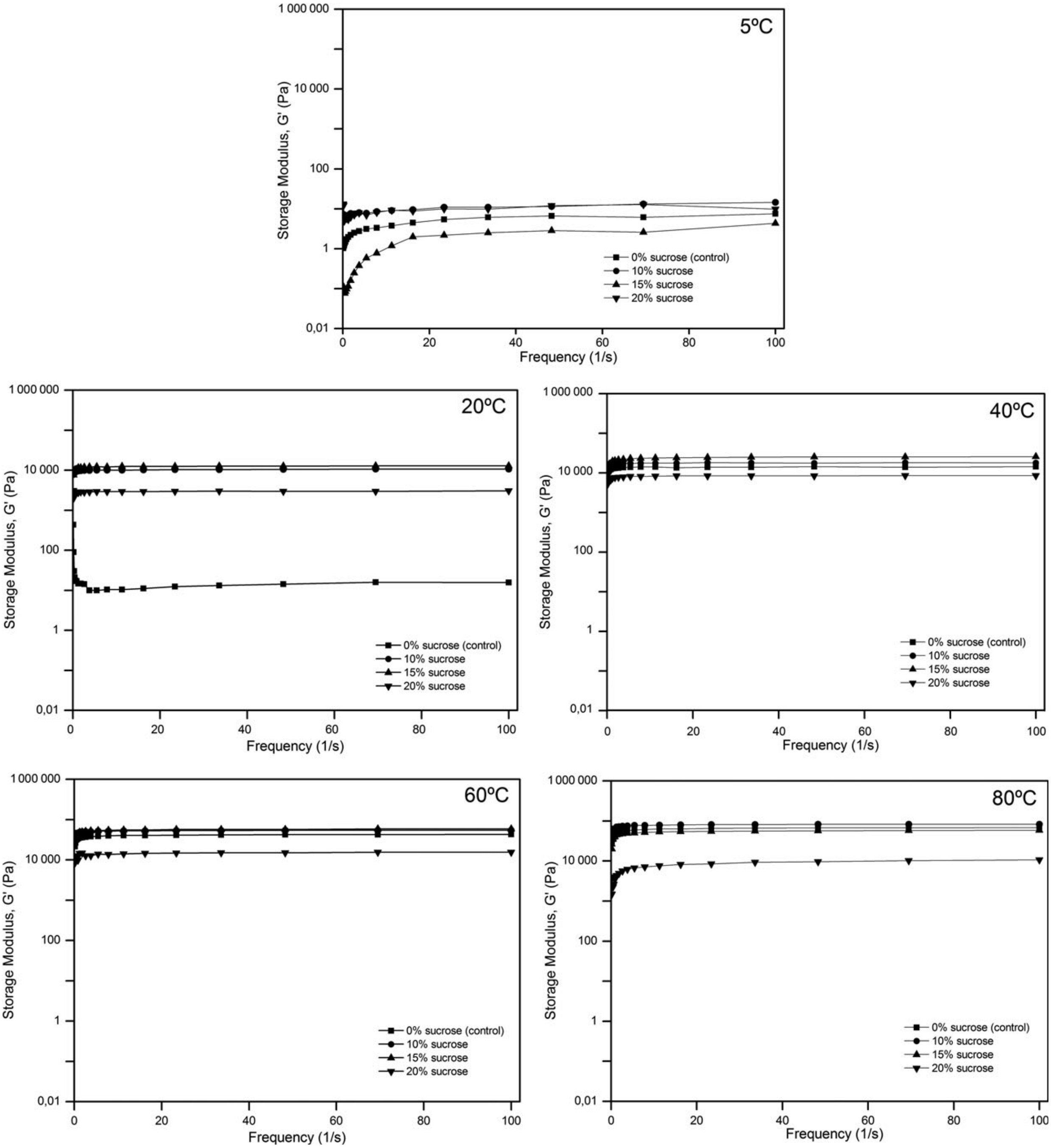

Figure 5 Effect of sucrose concentration on storage modulus (G') of nopal mucilage dispersions at: (a) 5 °C, (b) 20 °C, (c) 40 °C, (d) 60 °C and (e) 80 °C.

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modulus increased more than at the 10% concentration; however, at 80 °C, G' modulus exhibited no further increase, and there was no significant difference between this value and the control without sucrose. With a high sucrose concentration (20% w/v), G' modulus increased at low temperatures but when the temperature increased over 40 °C, the gel structure started to destabilise and G' modulus decreased to less than control values. The effect of sugar on the gel viscoelastic properties can be attributed to hydrogen bonds between the polymer and sugar-OH groups in which sucrose may create junction zones and stabilise the structure of the gel (Bayarri *et al.*, 2004). Despite this, later destabilisation is caused by the competition between sucrose and the gum for available water in the system and immobilising the water needed to conform junction zones (Torres *et al.*, 2013).

The effect of sweeteners on hydrocolloid viscoelasticity has been studied by several authors. While some hydrocolloids decrease their G' and G'' modulus by adding sucrose (Torres *et al.*, 2013), other authors reported that adding sweetener could increase or not affect these parameters (Bayarri *et al.*, 2004). The effect of sucrose on the gel matrix depends on the type of sweetener, type of hydrocolloid, temperature and concentration of both the gum and sweetener. According to Bayarri *et al.* (2004), G' and G'' modulus increased with an increasing sucrose concentration for dilute k-carrageenan gels (0.3%, w/w), but they remained constant when adding sucrose to concentrated gels (1.2%, w/w). This appears to be due to similar phenomena but taking place at lower concentrations.

Conclusion

Our findings show that nopal mucilage and *Prosopis chilensis* seed gum dispersions behave as weak gels (G' > G''), and their viscoelastic properties are highly dependent on temperature, and G' modulus increases as the temperature increases for both hydrocolloids. The addition of $CaCl_2$ does not follow a clear trend, and its effect depends on both temperature and gum concentration; however, the highest G' values were obtained by adding $CaCl_2$. As for sucrose, a low sucrose concentration could enhance viscoelasticity, but a high sucrose concentration destabilises the gel structure because the hydrocolloid and sucrose compete for the water available in the system. In the present study, we used an acid extraction method that is different from the alkaline extraction method used in previously published studies on nopal mucilage and *Prosopis chilensis* seed gum dispersions. It provides a different hydrocolloid structure that led to new behaviours for these two dispersions. In general, nopal mucilage and *Prosopis chilensis* seed gum dispersions maintain the weak gel structure even at high temperatures, thus making it a promising ingredient in food applications that require heat tolerance.

Conflict of interest

The authors declare no conflict of interest, in terms of scientific, financial and personal.

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