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Native hybrid carrageenans from *Sarcopeltis skottsbergii* and *Sarcothalia crispata*: the role of counterions on their gelling properties

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Abstract

Carrageenans are red seaweed polysaccharides of great importance as gelling agents in the food texture market. The hybrid kappa/iota carrageenan is extracted from certain cold-water species and its unique properties have given rise to growing demand for this biopolymer. Gelation of these polysaccharides is affected by the valency and, sometimes, the identity of counterions. The main objectives of this work were to investigate the role of potassium and calcium ions on the gelation of the native hybrid carrageenans from *Sarcopeltis skottsbergii* and *Sarcothalia crispata* from the South American Atlantic coast, and to optimize the structural properties of these gels. Response surface methodology was used to analyze the effects of carrageenan concentration, salt concentration, and KCl:$CaCl_2$ ratio, on the rheology, texture, syneresis after 50% deformation, and color of these gels. Both native carrageenans presented high extraction yield (42.73 ± 0.20 g/100 g for *S. skottsbergii* and 20.43 ± 7.64 g/100 g for *S. crispata*) and formed either weak or strong gels upon the addition of salt. Carrageenan and salt concentrations increased gel strength, but also increased turbidity. Moreover, KCl fraction also increased gel strength, attributed to the predominance of the kappa diad in these carrageenans, whose gelation is favored by potassium ions. All samples showed syneresis after deformation, being minimized with higher carrageenan and salt contents. Optimal compositions predicted by the selected models to maximize gel strength and minimize syneresis and haze, were 1.13% carrageenan, 0.39 M salt and a proportion of 0.85 KCl for *S. skottsbergii*, and 1.16% carrageenan, 0.36 M salt and a proportion of 0.85 KCl for *S. crispata*. Predicted responses for these optimal compositions were in accordance with the observed responses. Considering the worldwide growing demand for kappa/iota-carrageenan in the food industry, *S. skottsbergii* and S. *crispata* from the South Atlantic represent a potential source of these biopolymers.

Keywords: κ/ι-carrageenan, cation, rheology, texture

1. Introduction

Carrageenans are one of the principal hydrocolloids in the food texture market, where they are employed as gelling, stabilizing and thickening agents [1]. Present in the cell wall matrix of red seaweeds of the order Gigartinales, carrageenans are sulfated galactans consisting of linear chains of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose residues [2]. They are classified into different ideal disaccharide repeating units or diads, according to the sulfation pattern and the presence/absence of 3,6-anhydrogalactose in the 4-linked residue [3,4]. In addition, these structures influence the gel-forming ability: 3,6-anhydrogalactose is essential for gel formation while the presence of sulfate groups in certain positions impairs the development of the gel-network [5].

Three carrageenan types are of the greatest commercial relevance: kappa (κ-), iota (ι-) and lambda (λ-) [6], conferring different properties on preparations: κ-carrageenan forms firm and brittle gels, ι-carrageenan gels are soft and elastic, and λ- carrageenan does not gel but is used as a stabilizer [7,8]. In addition, the non-gelling mu (μ-) and nu (ν-) types, which are the biological precursors of κ- and ι-carrageenan, respectively, are found in native carrageenans [3] (carrageenans extracted without modification [2]). Alkaline treatment with high temperature during extraction is usually employed to convert μ- and ν- carrageenans into κ- and ι- types, respectively [9]. However, these procedures increase extraction costs and generate large amounts of effluents as a result of the use of alkali [10].

In nature, carrageenans usually contain mixtures of the mentioned idealized structures, with variations between seaweed species and between different life cycle phases of the same species [11]. κ- and ι-carrageenan are extracted from the tropical *Kappaphycus alvarezii* and *Eucheuma denticulatum*, respectively [7]. λ -carrageenan is extracted from the sporophytic phase of some cold-water species such as *Sarcopeltis skottsbergii* (ex-*Gigartina skottsbergii*), *Sarcothalia crispata* and *Mazzaella laminarioides* from the Southern hemisphere, and *Chondrus crispus* from the Northern hemisphere [12]. The gametophytic phase of these cold-water seaweeds produces copolymers of κ- and ι-carrageenan in different ratios, known as “kappa-2 carrageenan” or “κ/ι-hybrids” [12,13]. The properties of these κ/ι-hybrids are different from those of physical (hand-made) mixtures of κ- and ι-carrageenan [11,14,15] and there is great demand for them in some areas of the food industry because of their particular textural functionality and protein reactivity [1].

The currently accepted mechanism for gelation in carrageenans involves a coil-to-helix transition followed by aggregation of helices [16-18]. This process is significantly affected by the quantity and valence of counterions and, in some cases, also by their identity [18,19]. Specific ions, such as potassium (K^+), cesium (Cs^+), rubidium (Rb^+) and ammonium (NH_4^+), are effective helix stabilizers and also promote gelation of κ-carrageenan [18]. This cation specificity has been ruled out for pure ι-carrageenan [20]; however divalent cations have been found to be stronger helix-stabilizers than monovalent cations in this type of carrageenan [21,22]. The effect of individual cations on the gelation of mixtures of κ- and ι- carrageenan has been thoroughly studied [23–27]. In addition, synergism has been demonstrated when combining salts for the gelation of κ-carrageenan [28–30]. However, very few studies have been carried out on the effects of using combinations of salts on the gelation of hybrid or mixed κ- and ι- carrageenan [6,31]. In this matter, since κ/ι-carrageenan is a copolymer consisting of blocks of κ- and ι-disaccharide units [13], it would be expected for a mixture of a specific monovalent salt and a divalent salt to be more effective for inducing gelation than a single type of salt. In addition, considering the above mentioned disadvantages of using alkali treatment with high temperatures in the extraction process, it would be of interest to determine the range of gel properties that could be obtained for native hybrid carrageenans by using combinations of cations.

The aims of this study were to 1) evaluate the effect of calcium and potassium chloride on the rheology, texture, syneresis and color of native hybrid carrageenan gels from *Sarcopeltis skottsbergii* and *Sarcothalia crispata* gametophytic fronds from the South American Atlantic coast, and 2) optimize the structural properties of these gels by variations in carrageenan and salt concentrations and salt composition.

2. Materials and Methods

2.1. *Materials*

Sarcopeltis skottsbergii and *Sarcothalia crispata* gametophytic fronds were collected from the subtidal zone near Cabo Raso (44°18’42” S, 65°15’22” W), Chubut, Argentina. The life history phase of each frond was confirmed by the resorcinol test [32]. A disc (5 mm diameter) of each seaweed frond was placed in a test tube and 2 mL of the resorcinol-acetal reagent was added. Then the test tube was heated to 80 °C for 1 min in a water bath and the color of the reaction was recorded: pink-red indicates that it is a gametophyte and lack of color indicates the frond is a sporophyte. Gametophytes were separated, washed with filtered seawater, rinsed with distilled water and air dried on absorbent paper. Dried fronds were milled and sieved (10 mesh, ASTM) and the resulting powder was later used for carrageenan extraction.

Analytical grade potassium chloride (KCl) was obtained from Anedra (San Fernando, Buenos Aires, Argentina) and calcium chloride ($CaCl_2$) from Cicarelli (San Lorenzo, Santa Fe, Argentina).

2.2. Polysaccharide extraction and composition

Carrageenan extraction was carried out according to Matulewicz and collaborators [33]. Distilled water (2.5 L) was added to the milled seaweed (50 g) which was left under mechanical stirring (180 rpm) for 24 h at room temperature (25 °C). The algal residue was subsequently removed by centrifugation (3000 ×*g*, 15 °C, 15 min) and the polysaccharide was precipitated by pouring the supernatant in three volumes of isopropyl alcohol with continuous stirring. The liquid was decanted, the precipitate was pressed in filter paper, dried by solvent exchange and finally *in vacuo* at room temperature until constant weight.

For each species, the native polysaccharide of two (*S. skottsbergii*) or four (*S. crispata*) replicate extractions were pooled together for the analysis. Extraction yields were calculated as grams of carrageenan obtained per 100 grams of milled seaweed and expressed as g/100g.

Chemical characterization of the native polysaccharides from the gametophytic phase of *S. skottsbergii* and *S. crispata* had been carried out in a previous study [34]. Salt composition of carrageenan samples was determined by inductively coupled plasma-atomic emission spectrometry (ICP-OES) in a Shimadzu ICPE-9000 spectrometer (Shimadzu Corporation, Kioto, Japan). The amounts of K^+, Na^+, Ca^{2+} and Mg^{2+} were expressed as g/100g.

2.3. *Sample preparation*

Salt and carrageenan solutions were prepared according to the experimental design described in section 2.5. The corresponding amount of salt (KCl, $CaCl_2$ or both) was added to distilled water in a beaker and dissolved while stirring for five minutes at room temperature. The appropriate amount of carrageenan was then added, and the solution was heated at 80 °C in a water bath during 2 h under continuous stirring to guarantee carrageenan dissolution. The pH of the carrageenan-salt solution varied between 6 and 9 (at 30 ± 5 °C), assuring no hydrolysis of the carrageenan took place.

2.4. *Measurements*

2.4.1. Rheological properties

To study the viscoelastic properties of the different carrageenan gels, small deformation dynamic oscillatory measurements were performed in a Paar Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria), using cone-plate geometry (diameter = 50 mm, cone angle = 1°). Approximately 3 mL of the sample at 80 °C were placed in the rheometer plate, previously set at the same temperature; the cone was then lowered and the excess sample was removed. The exposed borders of the sample were covered with silicon oil and the measuring system was enclosed in a water trap in order to avoid dehydration. The system was allowed to rest for 5 min before measurements began. Storage (G') and loss (G") moduli were determined for each sample, during three successive steps: 1) cooling ramp: from 80 °C to 20 °C, at a cooling rate of 2 °C/min and a frequency of 1 Hz; 2) frequency sweep: from 100 to 0.01 Hz, at 20 °C; 3) heating ramp: from 20 °C to 80 °C at a heating rate of 2 °C/min and a frequency of 1 Hz. All measurements were taken at an amplitude strain of 0.5%, which was within the linear viscoelastic range (LVR). The LVR was determined in preliminary essays, which consisted of strain amplitude sweeps (from 0.01 to 100%, at 20 °C and 1 Hz) for each type of carrageenan.

2.4.2. Mechanical properties

Mechanical properties of the carrageenan gels were determined by a penetration test designed for pectin gels (TA-XTPlus Application Study GL3/P05R, Stable Micro Systems Ltd.) with modifications. The test was carried out with a TA-Plus texture analyzer (Lloyd Instruments Ltd., Bognor Regis, UK) equipped with a 50 N load cell, using a 25.5 mm diameter Teflon cylindrical probe with a flat end. The hot sample (80 °C) was poured into a cylindrical glass jar (65 mm diameter) up to a height of 25 mm, left to cool down for 1 h at room temperature, then covered and stored for 24 h at 4 °C. The gel was conditioned for 1 h at room temperature before the test, in which the probe penetrated to the center of the gel 12.5 mm (50% its depth) at a speed of 1 mm/s, after which it was withdrawn from the sample. Three parameters were determined from each force-time curve: rupture strength [N]: force at the first break; brittleness [mm]: distance to the first break (the shorter the distance, the more brittle the gel); and hardness [N]: maximum force.

2.4.3. Syneresis

Syneresis was determined as the water lost by the gels during the 24 h following the penetration test. In the mentioned test, gels were subjected to a deformation of 50% their height and, therefore, they had a damaged structure, resembling the process gelled foods go through during domestic use [35]. The gel used in the penetration test was weighted and put in a Büchner funnel with filter paper on its base, covered with film and placed on an Erlenmeyer flask. The gel was left 24 h at room temperature and then weighed again. Syneresis was calculated as the difference between the initial and final weight of the gels, according to Eq. 1:

$$Syneresis\ (\%) = \frac{m_i - m_f}{m_i} \times 100 \quad (1)$$

Where m_i is the initial weight of the gel after the penetration test and m_f the final weight of the gel after 24 h of water loss.

2.4.4. Color

Color parameters were measured in a HunterLab UltraScan XE color measurement spectrophotometer (Hunter Associates Laboratory Inc., Reston, USA). The hot sample was poured into a glass cell of 10 mm depth and conditioned for 1 h at room temperature. Parameters were measured in CIE L*a*b* scale: L* or luminosity (0 = black, 100 = white); a* (green (-), red (+)); b* (blue (-), yellow (+)); and haze (0 = clear, 100 = turbid). Both sides of the cell were measured for each sample and the values were averaged.

2.5. Experimental design and statistical analysis

Data were analyzed by response surface methodology, small composite design, with three factors: carrageenan concentration (Carr [% (w/w]), salt concentration (Salt [M]), and KCl fraction in a mixture of KCl and $CaCl_2$ (X_{KCl} = KCl / (KCl + $CaCl_2$)) (Table 1). This design was carried out for both types of carrageenan (*S. skottsbergii*, *S. crispata*) in order to evaluate differences in their gelling conditions. The evaluated responses were: elastic modulus at a frequency of 1 Hz during the frequency sweep (G'_{1Hz}), rupture strength (R), brittleness (B), hardness (H), syneresis (S), luminosity (L*) and haze (Z). Data, with two replicates and three central points, were analyzed with the Design Expert 10.0.1 software (Stat-Ease Inc., Minneapolis, USA).

3. Results and discussion

3.1. Carrageenan yield and characterization

Carrageenan extraction yields from *Sarcopeltis skottsbergii* and *Sarcothalia crispata* were 42.73 ± 0.20 g/100 g and 20.43 ± 7.64 g/100 g, respectively. These values are similar to those reported for *S. skottsbergii* [33], and *S. crispata* [36] using comparable extraction methods. Although other authors reported that higher yields were obtained with extractions at higher temperature compared to extraction at room temperature [27], yields obtained in the present work for *S. skottsbergii* were similar to those reported for this species at high temperatures [27].

The native carrageenans extracted from the gametophytic phase of *S. skottsbergii* (SS) and S. *crispata* (SC) were characterized in a previous study, and consisted of 70% κ-diad, 12% ι-diad and 18% ν-diad in *S. skottsbergii*, and 56% of κ-diad, 20% ι-diad and 24% ν-diad in *S. crispata* [34].

The salt composition in SS and SC was similar, consisting mainly of Na^+ and secondly K^+: 1.12 g/100g K^+, 3.14 g/100g Na^+, 0.32 g/100g Ca^{2+} and 0.70 Mg^{2+} in SS, and 1.45 g/100g K^+, 3.04 g/100g Na^+, 0.34 g/100g Ca^{2+} and 0.79 g/100g Mg^{2+} in SC.

3.2. Structural properties of carrageenan gels

3.2.1. Rheology

For all carrageenans and sample formulations, both the storage modulus (G') and the loss modulus (G") increased during the cooling ramp (from 80 to 20 °C). It has been reported for physical mixtures of κ- and ι- carrageenan a characteristic two-step gelation, as a result of the gelation of ι-carrageenan at higher temperatures and of κ-carrageenan at lower temperatures [31,38]. However, this two-step gelation process was not observed in the cooling ramp of SS or SC, nor has it been observed by other authors in hybrid carrageenans in the presence of KCl [27,39], $CaCl_2$ [27] or NaCl [27,40]. It has been proposed that hybrid κ/ι-carrageenans are formed by κ- and ι- disaccharide units coexisting in the same polysaccharide chain, unlike physical mixtures of κ- and ι-carrageenan [13,15], which may explain the absence of two step gelation during cooling ramps. However, two-step gelation was indeed reported for hybrid carrageenan in the presence of NaCl using a wide range of ionic strengths [41]. Therefore, further studies may be needed to elucidate hybrid carrageenan gelling process.

Fig. 1 shows the cooling ramps of representative samples with different behaviors, corresponding to two carrageenan concentrations (1.1 and 2%), two salt concentrations (0 and 0.25 M) and equal proportions of KCl and $CaCl_2$. SS (Fig. 1A) and SC (Fig. 1B) gels showed a similar behavior, which varied according to the composition of the samples. In most cases, G' was higher than G" during the entire temperature ramp, as can be observed for the formulation with Carr = 1.1%, Salt = 0.25 M and X_{KCl} = 0.5. This indicates a predominantly solid-like behavior of these samples in the temperature range evaluated [42]. There was only one sample, corresponding to the combination of factors with no added salt (Carr = 1.1%, Salt = 0 M), in which G' was lower than G" throughout the temperature ramp. This means that the solution did not gel and indicates that the addition of certain amount of salt is crucial for the gelation of the native carrageenans from *S. skottsbergii* and *S. crispata*, confirming what has already been reported as a requisite for the formation of carrageenan gels [16]. There were three combinations of factors (Carr = 1.7%, Salt = 0.07 M, X_{KCl} = 0.85; Carr = 1.7%, Salt = 0.43, X_{KCl} = 0.15 and Carr = 2%, Salt = 0.25 M, X_{KCl} = 0.5), all with high carrageenan concentrations, in which G' was lower than G" at high temperatures and higher at low temperatures, giving rise to a crossover of the G'-G" curves. In gel systems, this crossover is considered to indicate the gel point (GP) or gel setting temperature [43]. In the present study, the GP varied between 26 and 59 °C for SS and between 39 and 54 °C for SC systems. These gel setting temperatures are similar to those recently reported for the native carrageenan from *S. skottsbergii,* which varied between 42 and 64 °C using different salts [27], and also for the κ/ι- carrageenan from *Mastocarpus stellatus*, which ranged between 33 and 52 °C for similar biopolymer and salt concentrations but with NaCl [40]. This method for obtaining the GP of carrageenans has been employed in many studies [27,44–47]. However, in the hybrid carrageenan systems analyzed in the present work, the crossover of the G'-G" curves only occurred for some carrageenan and salt combinations, whereas in the majority of the samples G' was higher than G" throughout the heating ramp, as also reported for other gelling systems [48]. In addition, the G'-G" crossover method for obtaining the GP in the gelation of biopolymers has certain limitations [43]. Regarding this, a sound method for determining the formation of the carrageenan gel network based on the correlation between shear modulus and strain-optical coefficient has been stablished [49].

During the frequency sweep (100 - 0.01 Hz) for both types of carrageenan, G' was higher than G" (one or two orders of magnitude, depending on sample composition) through the entire interval for all the samples except the ones with no added salt, in which, G' was lower than G" for all frequencies evaluated (not shown), which indicates a predominant liquid-like behavior [42]. Although a certain amount of salt was already present in the native polysaccharides extracted from *S. skottsbergii* and *S. crispata*, it was evidently not sufficient to trigger gel formation. This was confirmed by the computation of total ionic strength contributed by these initial amounts of salts, which was negligible compared to the total ionic strength of the other samples with added salt. In addition, the main cation present in SS and SC was Na^+, which is not as effective as K^+ and Ca^{2+} for the gelation of κ- and ι-carrageenan, respectively [21].

Fig. 2 shows the effect of the different factors on G' throughout the frequency sweep. In both carrageenans, G' was almost frequency-independent (typical of strong gels) for carrageenan and salt concentrations higher than 1.1% (Fig. 2A) and 0.25 M (Fig. 2B), respectively, and salt composition consisting mainly of KCl (more than 50% of the total added salt) (Fig. 2C). Otherwise, G' varied along the frequency interval, particularly at high frequencies, which is typical of weak gels. In strong gels, the molecular rearrangements within the network are very reduced over the time scales analyzed, G’ is higher than G” throughout the frequency range, and is almost independent of the frequency; in weak gels, there is a higher dependency on frequency for the dynamic moduli, suggesting the existence of relaxation processes occurring even at short time scales, and a smaller difference between G’ and G”, indicating that a lower percentage of the stored energy is recovered [43]).

In order to statistically compare the samples, the value of G’ at a frequency of 1 Hz (G'_{1Hz}) was selected as representative of the gel strength of each sample (Table 2). The three evaluated factors had significant effects on G'_{1Hz} of SS and SC: an increase in carrageenan concentration, salt concentration or KCl fraction, increased G'_{1Hz}. The effect of counterions on the gelation of κ- or ι-carrageenan has been thoroughly studied and the specificity of κ-carrageenan for binding certain monovalent cations such as K^+ has been demonstrated [18,19,29] as well as the higher efficiency of Ca^{2+} to stabilize ι-carrageenan helices [21,50]. Although SS and SC consist of κ-, ι-, and ν- diads, the predominant structure is the κ- diad (70% in SS and 56% in SC) [34], which might explain the positive effect of KCl compared to $CaCl_2$. Even though the gelling mechanism of hybrid carrageenans has yet to be fully understood, it is well accepted that, in order to form gels, there has to be a coil to helix transition induced by the presence of cations, followed by aggregation of helices with further cooling [16,17]. As it was mentioned, there is evidence that native hybrid carrageenans consist of blocks of κ- and ι-structures interrupted by μ- and ν- disaccharide units [13,51]. Therefore, since native hybrid carrageenans from *S. skottsbergii* and *S. crispata* formed gels, these blocks of κ- and ι-disaccharide units should have certain length to allow helix formation and subsequent aggregation [39].

The maximum G'_{1Hz} reached in this study was 712 ± 15 Pa for SS and 181 ± 54 Pa for SC, obtained at Carr = 1.1%, Salt = 0.25 M, $X_{KCl} = 1$ and Carr = 2%, Salt = 0.25 M, $X_{KCl} = 0.5$, respectively. These values were similar to the ones obtained for *S. skottsbergii* with 2% biopolymer content and 0.5 M $CaCl_2$ [27].

In order to analyze the thermoreversibility of the gels, they were subjected to a heating ramp from 20 to 80 °C, in which G’ and G” decreased with increasing temperature, as can be observed for SS and SC in Fig. 3A and B, respectively. However, these curves did not follow the exact same paths as those in the initial cooling ramp (from 80 to 20 °C). Kappa-carrageenan gels are known to present hysteresis, i.e. a difference in the gelling and melting temperatures, which is attributed to the gelling mechanism of these biopolymers [22]. On the other hand, pure ι- carrageenan does not show differences in gelling and melting temperatures [22], while κ/ι-carrageenan shows intermediate hysteresis between κ- and ι- types [27]. Hysteresis has been also reported by other authors for hybrid carrageenan containing precursor units [39], as is the case of the carrageenans studied in the present work. In addition, in some samples, a pronounced increase in G' and G'' was observed at the end of the heating ramp, as was the case with SS when Carr = 2%, Salt = 0.25 M and X_{KCl} = 0.5 (Fig. 3A). This behavior can be at least partially attributed to dehydration of the sample when reaching such high temperatures.

3.2.2. Texture

From the force-time curves (not shown), rupture strength (R), brittleness (B) and hardness (H) were obtained (Table 2). Carrageenan concentration, salt concentration and KCl fraction had positive effects on rupture strength and hardness of SS and SC gels, i.e. the higher amount of carrageenan, salt or KCl, the stronger the gels. The maximum hardness for SS gels was 0.72 ± 0.01 N (at Carr = 2%, Salt = 0.25 M and X_{KCl} = 0.5) and for SC gels 0.41 ± 0.03 N (at Carr = 1.1%, Salt = 0.25 M and X_{KCl} = 1). Thrimawithana and collaborators [40], found that ι-carrageenan gel strength was 0.3 times lower than that of κ-carrageenan, which is in agreement with the present results since SS has more κ- and less ι-content than SC.

The force-time curve of SS gels showed an early drop of the force when the carrageenan concentration was above or equal to 1.1%, which indicates the point where the gel breaks. This break only appeared in one sample of SC gels (Carr = 1.7%, Salt = 0.07 M and X_{KCl} = 0.85), indicating that these gels were softer or less brittle than SS gels. This difference was also attributed to the higher amount of κ- and lesser amount of ι- diads in SS compared to SC, since it is well known that κ- is more brittle than ι-carrageenan [7]. In the present study, an increase in carrageenan concentration, salt concentration and KCl fraction resulted in more brittle SS gels, i.e. the higher the level of these factors, the shorter the distance to the first break (lower B values). On the other hand, only carrageenan concentration had a significant effect on SC brittleness.

3.2.3. Syneresis

It is well known that κ-carrageenan gels produce syneresis (or water loss), unlike ι-carrageenan [22,52]. Although it has been reported that κ/ι-carrageenan gels are also syneresis free [52], in the present work all samples presented a certain amount of water loss that varied with sample composition. It must be clarified that no water loss by these gels was observed after rheological measurements were taken. Therefore, syneresis values reported for these gels may be a consequence of the deformation and breaking of the gels caused by the penetration test, since syneresis in this study was measured after mechanical tests were performed.

In SS and SC gels, syneresis after the penetration test decreased at increasing concentrations of both carrageenan and salt, while KCl fraction had no significant effect on this response (Table 3). The minimum syneresis, which was 8.22 ± 0.06% for SS and 6.76 ± 0.37% for SC gels, was achieved at the highest carrageenan concentration (2%). It was expected that a higher carrageenan concentration would entrap more water in the gel network and, therefore, syneresis would be minimized, as indeed occurred in the carrageenans evaluated.

3.2.4. Color

In SS and SC gels, a* values (not shown) were positive but close to zero (0.16 to 2.55 and 0.1 to 9.54, respectively), indicating that the samples were slightly red. Both types of carrageenan gels showed positive b* values (1.32 to 13.65 in SS, 2.05 to 21.03 in SC), indicating that the gels showed a yellowish color.

In general, luminosity (L*) varied between 79.0 ± 0.6 and 98.6 ± 0.1 (Table 3), indicating that the gels were light colored. For both types of carrageenan, L* was negatively affected by carrageenan and salt concentrations, and in SC it was positively affected by the KCl fraction. In this way, gels formulated with low carrageenan and salt concentrations and high KCl fractions where lighter. Haze (Z) varied between 27.1 ± 0.7 and 97.3 ± 0.1 among samples (Table 3), and was affected by carrageenan content in both carrageenans, and also by the salt concentration in SC. Therefore, the higher the carrageenan and salt concentrations, the hazier or cloudier the gels, due to the higher concentration of solids. Turbidity at high κ/ι-carrageenan concentration has been reported by other authors [27], as well as an increase in turbidity with increasing salt concentration in mixed κ- and ι-carrageenan systems [31]. This could be explained by increased aggregation of the system in the presence of higher biopolymer content and salt concentration.

3.3. Modelling, optimization and verification

The selected responses (rheological and mechanical properties, syneresis and color) of each type of carrageenan gel were modelled in terms of the three evaluated factors (carrageenan concentration, salt concentration and KCl fraction). The regression analyses suggested that the quadratic model best explained all the responses except SS hardness and rupture strength, where the two-factor interaction model was preferred (Table A1). The resulting response surface plots of G'_{1Hz}, H, S and Z are shown in Fig. 4A-D for SS and Fig. 4E-H for SC. In general, carrageenan concentration had a positive effect on G'_{1Hz}, H and R (it increased gel strength), a negative effect on syneresis (it decreased water loss), it reduced L* and increased Z (it made the gels darker and hazier). Salt concentration had a positive effect on G'_{1Hz}, H and R. It is important to point out that, for intermediate carrageenan concentration in SS, maximum G' was achieved with salt concentrations lower than the maximum tested (0.5 M), which is reflected on the corresponding surface plot (Fig. 4A), a behavior similar to the reported for ι-carrageenan gels in the presence of Ca^{2+} [52]. In addition, salt had a negative effect on S and L* in both types of carrageenan, and positively influenced Z in SC. KCl content had a positive impact on gel strength, increasing G'_{1Hz}, H and R. It also had a positive effect on L* in SC.

Although there is a wide range of carrageenan applications with different requirements in terms of gel properties, two possible combinations of desired responses were evaluated in this work: 1) giving priority to the syneresis, and the rheological and mechanical properties of the gels, and 2) taking into account all the evaluated responses. The formulation required to achieve each combination was determined by numerical optimization. In the first optimization, G'_{1Hz}, H, R and B were maximized while S was minimized. This gave the following factor combination: Carr = 1.74%, Salt = 0.32 M and

X_{KCl} = 0.52 with a desirability of 0.84 for SS, and Carr = 1.60%, Salt = 0.43 M and X_{KCl} = 0.40 with a desirability of 1 for SC. In the second optimization G'_{1Hz}, H, R, B and L* were maximized, and S and Z were minimized. The optimized factor combination was: Carr = 1.13%, Salt = 0.39 M and X_{KCl} = 0.85 with a desirability of 0.54 for SS, and Carr = 1.16, Salt = 0.36 and X_{KCl} = 0.85 with a desirability of 0.62 for SC.

The suitability of the chosen models for predicting the responses under specific factor combinations was verified. In order to achieve this, rheological, mechanical, syneresis and color measurements were performed at the combinations of factors determined in each optimization. These actual responses were compared with the response values predicted by the models for each type of carrageenan, verifying that they were within the 95% prediction interval. The actual response values were in agreement with the predicted values (Table 4), indicating the suitability of the chosen models for making predictions in the evaluated range of each factor.

Conclusions

Native hybrid carrageenans from *Sarcopeltis skottsbergii* and *Sarcothalia crispata* presented high extraction yields and were able to gel. Carrageenan gels from both seaweed species showed similar rheological and mechanical properties, which were improved with high biopolymer and salt concentrations and with high KCl:$CaCl_2$ ratio. The resulting gels were relatively hazy and showed variable syneresis, which could be minimized by increasing biopolymer and salt content. This study enabled to obtain gelling hybrid carrageenans from *S. skottsbergii* and *S. crispata* without the application of alkali treatment, and to optimize their gel properties by adjusting biopolymer and salt contents as well as salt composition. Considering the growing demand for κ/ι-carrageenan in some areas of the food industry, *S. skottsbergii* and *S. crispata* from the South American Atlantic coast could be considered a potential source of raw material for the extraction of these biopolymers.

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Tables

Table 1 Experimental design factors expressed in coded and actual levels. Carr: carrageenan concentration, Salt: salt concentration, X_{KCl}: potassium chloride fraction

Factor	-1.41	-1	0	1	1.41
Carr (% (w/w))	0.2	0.46	1.1	1.74	2
Salt (M)	0	0.07	0.25	0.43	0.5
X_{KCl}	0	0.15	0.5	0.85	1

Table 2 Elastic modulus at 1 Hz (G'_{1Hz}), rupture strength (R), brittleness (B) and hardness (H) of *S. skottsbergii* (SS) and *S. crispata* (SC) carrageenan gels for all the factor combinations of the selected design. Carr: carrageenan concentration (% (by mass)), Salt: salt concentration (M), X_{KCl}: potassium chloride fraction. Response values are expressed as mean ± standard error

Sample	SS				SC			
(Carr, Salt, X_{KCl})	G'_{1Hz} (Pa)	R (N)	B (mm)	H (N)	G'_{1Hz} (Pa)	R (N)	B (mm)	H (N)
0.2, 0.25, 0.5	0.60 ± 0.13	0.12 ± 0.00	12.5 ± 0.0	0.12 ± 0.00	0.67 ± 0.07	0.13 ± 0.00	12.5 ± 0.0	0.13 ± 0.00
0.46, 0.07, 0.15	0.57 ± 0.01	0.12 ± 0.00	12.5 ± 0.0	0.12 ± 0.00	0.63 ± 0.06	0.12 ± 0.00	12.5 ± 0.0	0.12 ± 0.00
0.46, 0.43, 0.85	16.9 ± 1.0	0.16 ± 0.00	10.7 ± 0.3	0.16 ± 0.00	3.55 ± 0.97	0.12 ± 0.00	12.4 ± 0.1	0.12 ± 0.00
1.1, 0, 0.5	1.61 ± 0.11	0.12 ± 0.00	12.4 ± 0.1	0.12 ± 0.00	1.58 ± 0.66	0.12 ± 0.00	12.4 ± 0.1	0.12 ± 0.00
1.1, 0.25, 0	7.22 ± 0.82	0.15 ± 0.00	12.5 ± 0.0	0.15 ± 0.00	2.24 ± 0.71	0.13 ± 0.00	12.5 ± 0.0	0.13 ± 0.00
1.1, 0.25, 0.5	229 ± 50	0.28 ± 0.02	10.6 ± 0.8	0.28 ± 0.02	27.8 ± 7.8	0.16 ± 0.01	12.5 ± 0.0	0.16 ± 0.01
1.1, 0.25, 1	712 ± 15	0.41 ± 0.01	4.69 ± 0.05	0.41 ± 0.01	91.3 ± 23.8	0.41 ± 0.03	11.6 ± 0.0	0.41 ± 0.03
1.1, 0.5, 0.5	101 ± 4	0.42 ± 0.01	8.77 ± 0.87	0.49 ± 0.08	83.7 ± 2.5	0.39 ± 0.06	12.5 ± 0.0	0.39 ± 0.06
1.74, 0.07, 0.85	583 ± 69	0.48 ± 0.01	3.23 ± 0.02	0.52 ± 0.01	95.7 ± 11.3	0.18 ± 0.01	6.25 ± 0.95	0.23 ± 0.01
1.74, 0.43, 0.15	164 ± 44	0.35 ± 0.01	9.91 ± 0.11	0.35 ± 0.01	52.8 ± 9.5	0.34 ± 0.01	12.5 ± 0.0	0.34 ± 0.01
2, 0.25, 0.5	629 ± 3	0.72 ± 0.01	7.36 ± 0.07	0.72 ± 0.01	181 ± 54	0.39 ± 0.04	9.92 ± 0.44	0.39 ± 0.04

Table 3 Syneresis (S), luminosity (L*) and haze (Z) of *S. skottsbergii* (SS) and *S. crispata* (SC) carrageenan gels for all the factor combinations of the selected design. Carr: carrageenan concentration (% (by mass)), Salt: salt concentration (M), X_{KCl}: potassium chloride fraction. Response values are expressed as mean ± standard error

Sample	SS			SC		
(Carr, Salt, X_{KCl})	S (%)	L*	Z	S (%)	L*	Z
0.2, 0.25, 0.5	94.6 ± 5.4	98.6 ± 0.1	27.6 ± 0.3	100 ± 0	98.3 ± 0.1	27.1 ± 0.7
0.46, 0.07, 0.15	100 ± 0	97.5 ± 0.5	46.9 ± 7.8	100 ± 0	96.1 ± 0.2	58.6 ± 0.5
0.46, 0.43, 0.85	18.8 ± 4.2	96.7 ± 0.0	54.5 ± 1.7	24.6 ± 1.4	95.6 ± 0.1	57.4 ± 0.3
1.1, 0, 0.5	74.6 ± 2.3	95.2 ± 0.1	80.7 ± 0.3	53.8 ± 0.6	95.0 ± 0.0	72.6 ± 0.4
1.1, 0.25, 0	12.5 ± 0.4	93.6 ± 0.2	80.1 ± 1.0	12.3 ± 1.7	90.1 ± 0.0	84.8 ± 1.5
1.1, 0.25, 0.5	15.7 ± 0.2	92.1 ± 0.2	84.5 ± 1.3	13.6 ± 2.5	89.6 ± 1.0	88.3 ± 0.6
1.1, 0.25, 1	12.7 ± 0.4	93.8 ± 0.1	81.8 ± 2.1	8.58 ± 0.73	93.6 ± 0.5	76.9 ± 2.4
1.1, 0.5, 0.5	11.6 ± 1.2	91.7 ± 0.0	85.2 ± 1.0	8.39 ± 1.58	89.0 ± 0.7	90.3 ± 0.2
1.74, 0.07, 0.85	11.0 ± 1.2	89.8 ± 0.3	93.2 ± 0.5	8.97 ± 1.22	86.8 ± 0.5	93.4 ± 0.5
1.74, 0.43, 0.15	9.82 ± 1.19	87.6 ± 0.1	93.9 ± 0.4	7.43 ± 1.14	81.9 ± 1.2	95.6 ± 0.3
2, 0.25, 0.5	8.22 ± 0.06	83.1 ± 1.2	96.6 ± 0.2	6.76 ± 0.37	79.0 ± 0.6	97.3 ± 0.1

Table 4 Predicted and actual values of each response for the two optimal factor combinations in *S. skottsbergii* (SS) and *S. crispata* (SC) carrageenan gels. G'$_{1Hz}$: elastic modulus, R: rupture strength, B: brittleness, H: hardness, S: syneresis, L*: luminosity, Z: haze

		Optimization 1		Optimization 2	
		SS	SC	SS	SC
G'$_{1Hz}$ (Pa)	Predicted	674	266	591	219
	Actual	563	152	140	129
R (N)	Predicted	0.724	0.532	0.581	0.431
	Actual	0.657	0.266	0.458	0.287
B (mm)	Predicted	7.30	12.5	6.07	12.1
	Actual	8.28	12.5	5.99	10.6
H (N)	Predicted	0.924	0.450	0.724	0.4[illegible]4
	Actual	0.657	0.266	0.458	0.287
S (%)	Predicted	8.30	6.40	8.5	6.60
	Actual	7.38	6.84	10.1	7.99
L*	Predicted	85.4	83.1	91.1	89.7
	Actual	86.8	84.0	91.8	90.0
Z	Predicted	97.8	100.0	87.8	91.6
	Actual	92.0	95.3	82.1	88.1

Table A1 Model coefficients in terms of the actual factors for each response and the corresponding adjusted R^2 of each model**.** Asterisks next to the coefficients indicate statistical significance according to ANOVA results: $p \leq 0.05$ (*), $p \leq 0.01$ (**) or $p \leq 0.001$ (***). Carr: carrageenan concentration (% (w/w)), Salt: salt concentration (M), X_{KCl}: potassium chloride fraction, G'_{1Hz}: elastic modulus (Pa), R: rupture strength (N), B: brittleness (mm), H: hardness (N), S: syneresis (%), L*: luminosity, Z: haze

Carrageenan	Response	Coefficients										R^2_{Adj}
		Intercept	Carr	Salt	X_{KCl}	Carr*Salt	Carr*X_{KCl}	Salt*X_{KCl}	$Carr^2$	$Salt^2$	X_{KCl}^2	
S. skottsbergii	$\sqrt{G'_{1Hz}}$	-0.729	-5.52***	32.8***	-6.10***	42.6***	22.8***	9.75	-1.37	-134***	2.60	0.976
	Log R	-0.833	-0.267*	0.546*	-0.515*	0.799*	0.871*	0.720*	0.029	-1.41*	-0.183*	0.993
	B	9.80	6.26***	-5.47***	10.1***	-3.77	-11.2***	11.0	-0.958	-1.92	-8.43***	0.959
	$1/\sqrt{H}$	2.84	0.375***	-2.07***	1.15**	-1.73***	-2.16***	-0.778	0.086	3.02**	0.397*	0.986
	Log S	2.68	-1.28***	-4.48***	1.35	1.56***	-0.399*	-1.05	0.229***	3.34***	-0.638**	0.973
	L*	99.7	-3.29***	-4.74***	1.55	-2.48	-2.33	-20.5***	-1.60**	21.4**	6.37***	0.985
	Z	5.61	100***	0.576	2.61	-9.92	-2.19	46.9	-26.4***	-8.57	-10.2	0.974
S. crispata	Log G'_{1Hz}	-0.672	0.297***	2.56***	-0.144***	2.87**	2.25***	0.193	-0.363*	-4.57*	-0.758	0.965
	Log R	-0.606	-0.519***	-1.59***	-0.078***	2.22***	0.465*	0.291	-	-	-	0.865
	B	11.3	3.88***	-12.8	7.83	11.3***	-6.65***	10.1*	-2.19***	-8.92	-3.95**	0.930
	H	0.290	-0.289***	-0.790***	-0.090***	1.13***	0.301*	0.167	-	-	-	0.815
	Log S	2.70	-1.35***	-3.79***	0.958	0.684	-0.512*	-0.317	0.355***	3.14**	-0.471*	0.963
	L*	99.4	-4.08***	-16.5***	6.20**	1.34	-3.39	-16.7	-2.38*	22.7	5.17	0.962
	Z	27.9	84.4***	8.68***	-57.9	-17.1	26.7**	105**	-24.8***	-14.0	-5.67	0.966

Fig. 1. Cooling ramp. Storage modulus (G') and loss modulus (G") at different temperatures during the cooling ramp of representative samples with different carrageenan and salt concentrations and equal proportion of potassium and calcium chloride. A) *S. skottsbergii* and B) *S. crispata* carrageenan. Carr: carrageenan concentration, Salt: salt concentration

Fig. 2. Frequency sweep. Storage modulus (G') during the frequency sweep of representative samples: A) Carrageenan concentration effect on G' with the rest of the factors at intermediate levels, B) salt concentration effect on G' with the rest of the factors at intermediate levels, C) KCl fraction effect on G' with the rest of the factors at intermediate levels. SS: *S. skottsbergii* carrageenan, SC: *S. crispata* carrageenan, Carr: carrageenan concentration, Salt: salt concentration, X_{KCl}: potassium chloride fraction

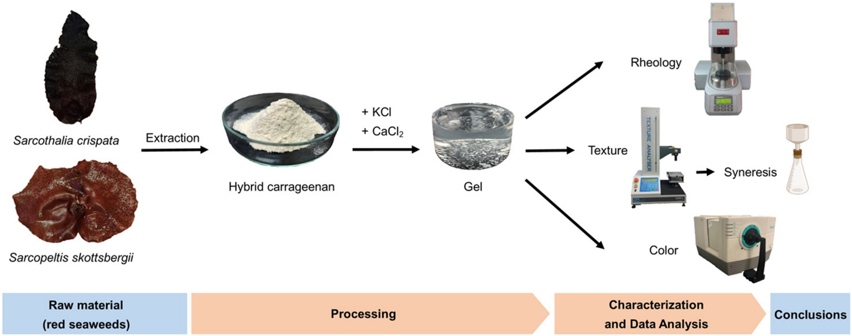
Fig. 3. Heating ramp. Storage modulus (G') and loss modulus (G") at different temperatures during the heating ramp of representative samples with different carrageenan and salt concentrations and equal proportion of potassium and calcium chloride. A) *S. skottsbergii* and B) *S. crispata* carrageenan. Carr: carrageenan concentration, Salt: salt concentration

Fig. 4. Response surface plots for selected properties of A-D) *S. skottsbergii* and E-H) *S. crispata* carrageenan gels. A,E) G'_{1Hz} and B,F) hardness as a function of salt concentration and potassium chloride fraction with carrageenan concentration at intermediate level. C,G) syneresis and D,H) haze as a function of carrageenan and salt concentrations with potassium chloride fraction at intermediate level. Carr: carrageenan concentration, Salt: salt concentration, X_{KCl}: potassium chloride fraction

Graphical abstract

Highlights

- Native hybrid carrageenans from both studied species produced weak but true gels.
- Biopolymer and salt contents increased the strength of native carrageenan gels.
- KCl fraction favored gel strength due to predominance of κ-diad over ι-diad.
- Syneresis after deformation was minimized with high carrageenan and salt contents.
- Optimal biopolymer and salt composition for best gel properties were estimated.



Graphics Abstract

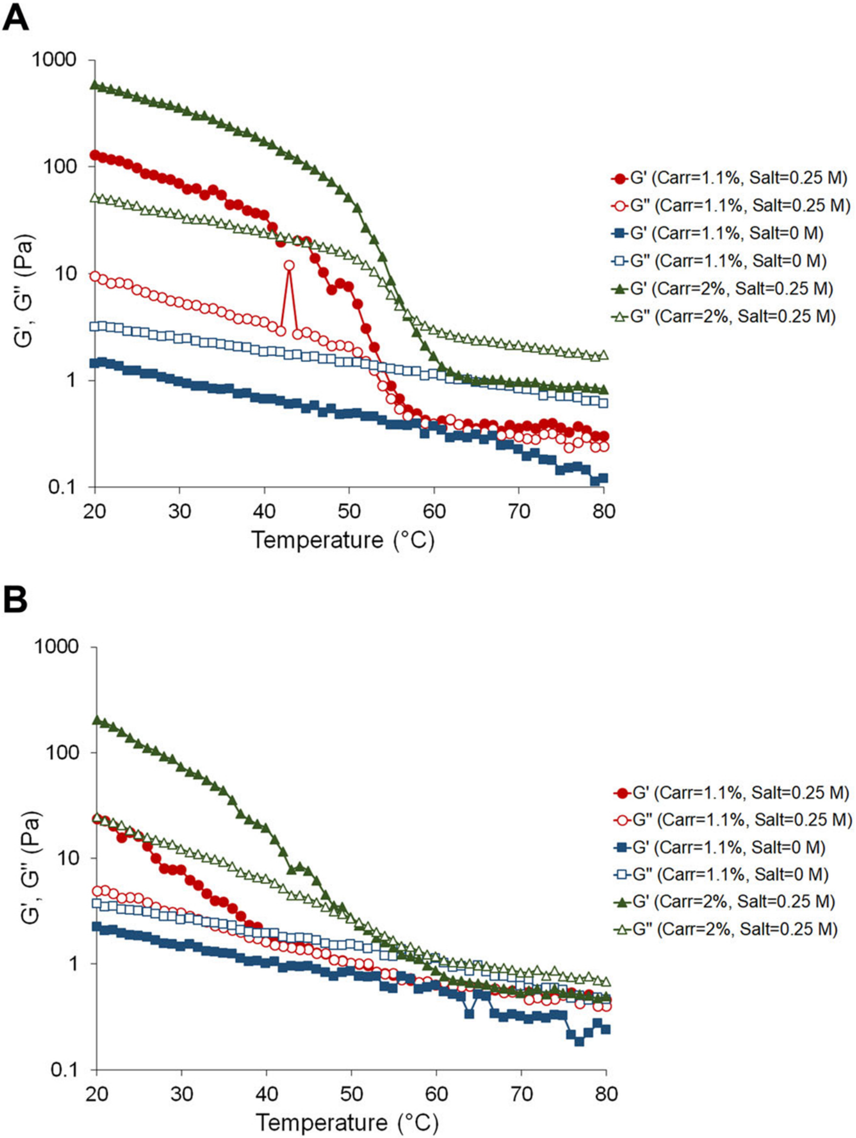

A
B
1000
100
10
1
0.1
20
30
40
50
60
70
80
G', G'' (Pa)
Temperature (°C)
G' (Carr=1.1%, Salt=0.25 M)
G'' (Carr=1.1%, Salt=0.25 M)
G' (Carr=1.1%, Salt=0 M)
G'' (Carr=1.1%, Salt=0 M)
G' (Carr=2%, Salt=0.25 M)
G'' (Carr=2%, Salt=0.25 M)

Figure 1

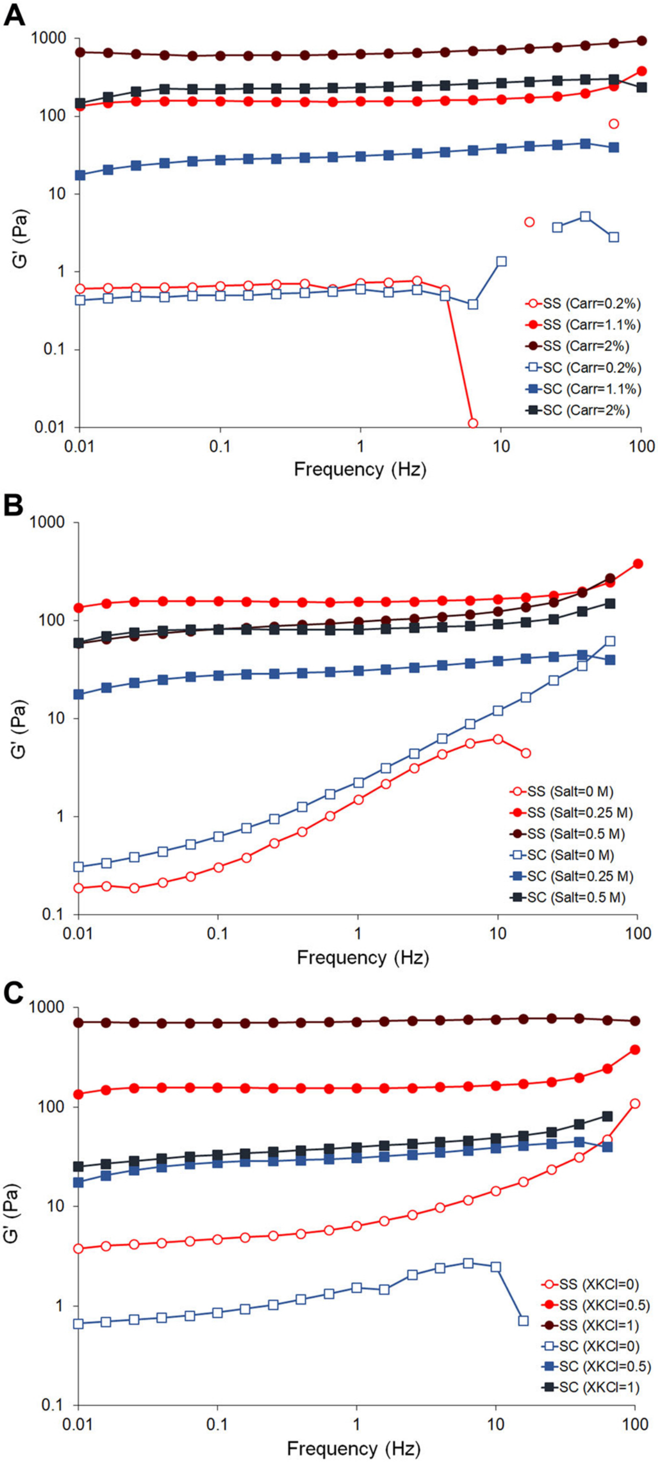

A
1000
100
10
1
0.1
0.01
G' (Pa)
0.01
0.1
1
10
100
Frequency (Hz)
SS (Carr=0.2%)
SS (Carr=1.1%)
SS (Carr=2%)
SC (Carr=0.2%)
SC (Carr=1.1%)
SC (Carr=2%)
B
1000
100
10
1
0.1
G' (Pa)
0.01
0.1
1
10
100
Frequency (Hz)
SS (Salt=0 M)
SS (Salt=0.25 M)
SS (Salt=0.5 M)
SC (Salt=0 M)
SC (Salt=0.25 M)
SC (Salt=0.5 M)
C
1000
100
10
1
0.1
G' (Pa)
0.01
0.1
1
10
100
Frequency (Hz)
SS (XKCl=0)
SS (XKCl=0.5)
SS (XKCl=1)
SC (XKCl=0)
SC (XKCl=0.5)
SC (XKCl=1)

Figure 2

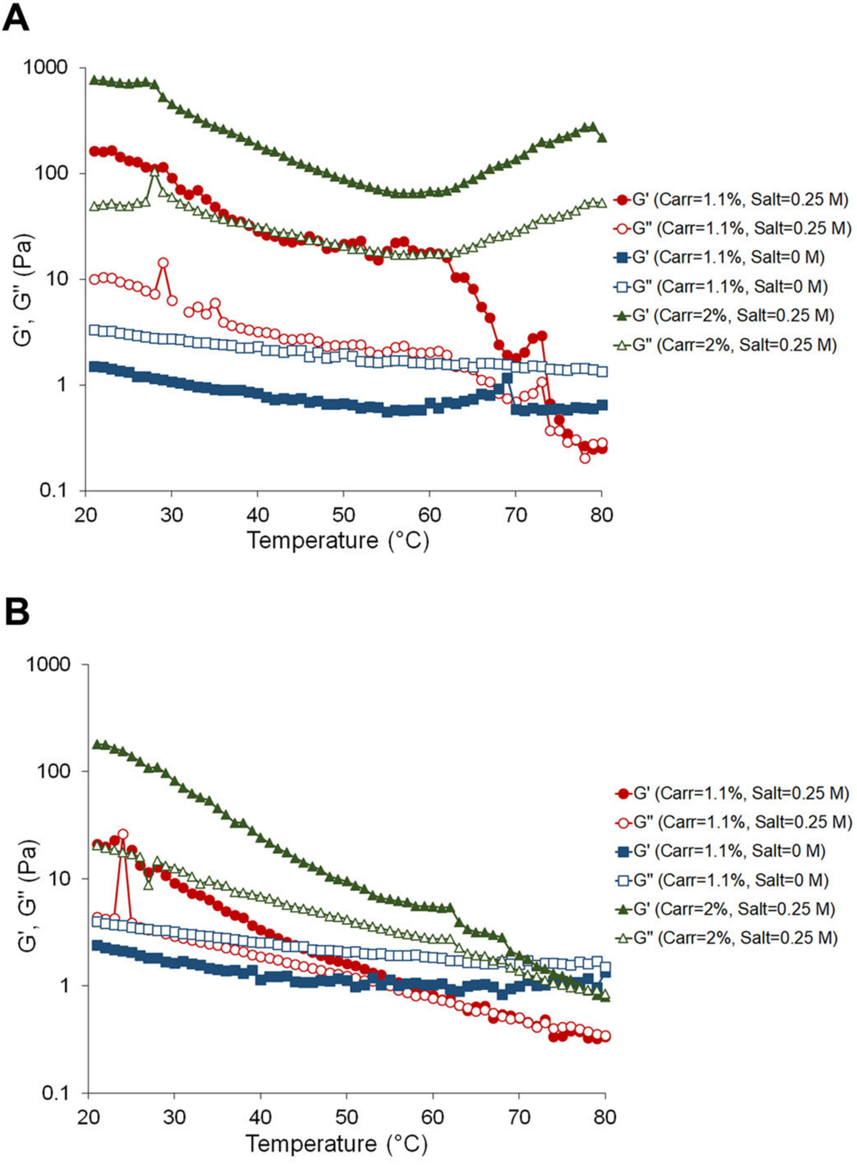

A
1000
100
10
1
0.1
G', G'' (Pa)
20
30
40
50
60
70
80
Temperature (°C)
G' (Carr=1.1%, Salt=0.25 M)
G'' (Carr=1.1%, Salt=0.25 M)
G' (Carr=1.1%, Salt=0 M)
G'' (Carr=1.1%, Salt=0 M)
G' (Carr=2%, Salt=0.25 M)
G'' (Carr=2%, Salt=0.25 M)
B
1000
100
10
1
0.1
G', G'' (Pa)
20
30
40
50
60
70
80
Temperature (°C)
G' (Carr=1.1%, Salt=0.25 M)
G'' (Carr=1.1%, Salt=0.25 M)
G' (Carr=1.1%, Salt=0 M)
G'' (Carr=1.1%, Salt=0 M)
G' (Carr=2%, Salt=0.25 M)
G'' (Carr=2%, Salt=0.25 M)

Figure 3

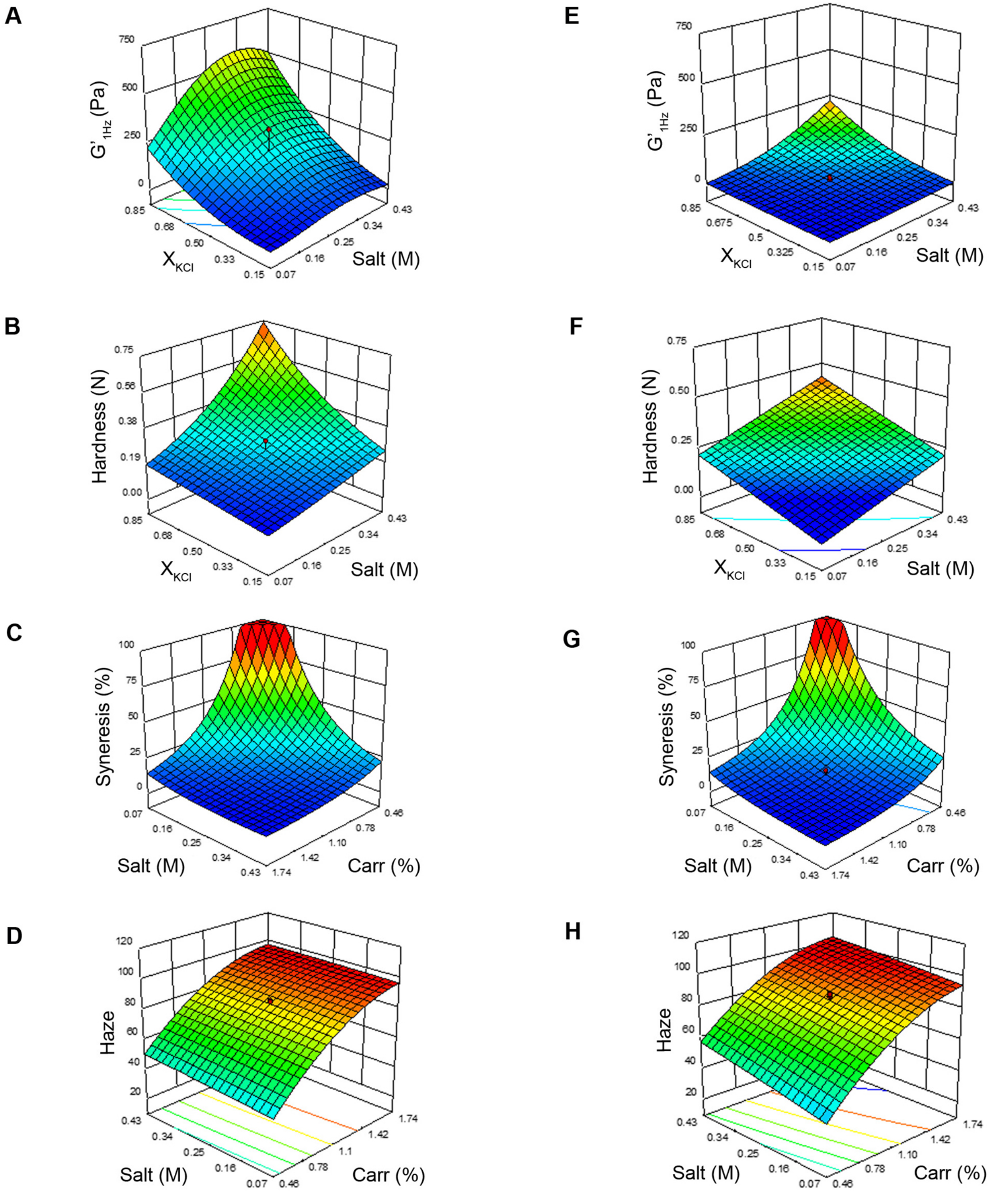

A
G'1Hz (Pa)
750
500
250
0
0.85
0.68
0.50
0.33
0.15
XKCl
0.07
0.16
0.25
0.34
0.43
Salt (M)
B
Hardness (N)
0.75
0.56
0.38
0.19
0.00
0.85
0.68
0.50
0.33
0.15
XKCl
0.07
0.16
0.25
0.34
0.43
Salt (M)
C
Syneresis (%)
100
75
50
25
0
0.07
0.16
0.25
0.34
0.43
Salt (M)
1.74
1.42
1.10
0.78
0.46
Carr (%)
D
Haze
120
100
80
60
40
20
0.43
0.34
0.25
0.16
0.07
Salt (M)
0.46
0.78
1.1
1.42
1.74
Carr (%)
E
G'1Hz (Pa)
750
500
250
0
0.85
0.675
0.5
0.325
0.15
XKCl
0.07
0.16
0.25
0.34
0.43
Salt (M)
F
Hardness (N)
0.75
0.50
0.25
0.00
0.85
0.68
0.50
0.33
0.15
XKCl
0.07
0.16
0.25
0.34
0.43
Salt (M)
G
Syneresis (%)
100
75
50
25
0
0.07
0.16
0.25
0.34
0.43
Salt (M)
1.74
1.42
1.10
0.78
0.46
Carr (%)
H
Haze
120
100
80
60
40
20
0.43
0.34
0.25
0.16
0.07
Salt (M)
0.46
0.78
1.10
1.42
1.74
Carr (%)

Figure 4