The temperature dependent extraction of polysaccharides from *Eucheuma* and the rheological synergistic effect in their mixtures with kappa carrageenan

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- Extraction
- Carrageenan
- Rheology
- Synergistic effect

**ABSTRACT**

Water extraction (at 60, 70, 80, and 90 °C) was applied to *Eucheuma* to explore the relationship between the extraction temperature and the structure of the extracted polysaccharides. The extracts mainly comprised iota carrageenan. The extraction temperature affected the compositions and properties of the polysaccharide extract. The yield decreased as the temperature increased (30.33–24.49%). Higher temperature extraction resulted in lower molecular height (60–90 °C: 0.57–0.48 nm) and layer height (60–90 °C: 0.76–0.58 nm) on atomic force microscopy images, leading to a better gel-forming potential; lower temperature extraction produced more diverse polysaccharides. 60 and 80 °C extracts were selected to represent low temperature extract and high temperature extract. Though the extracts had a weakness gelling ability, they cooperated with kappa carrageenan through synergistic effect. 80 °C extracts provided even higher synergistic effect than commercial IC (iota carrageenan) with stronger gel. It can be due to the amount of crosslink density variation. Possible schematic presentation was shown accordingly to illustrate the synergy.

**1. Introduction**

Recently, the production of seaweeds has increased dramatically and the nutrient value of them has attracted increasing attention (Kato, Hayashi, Umene, & Masunaga, 2016). In particular, Asian populations have long consumed red seaweeds as food (Pereira, Amado, Critchley, van de Velde, & Ribeiro-Claro, 2009). Plenty of polysaccharides from seaweeds are hydrocolloids frequently used to tune rheological properties of food systems. Carrageenan is a group of water-soluble anionic sulphated polysaccharides extracted from red seaweeds (Rhim, 2012). Thermo-reversible gels can be produced by two types of carrageenan: κ carrageenan (KC) and ι carrageenan (IC) (Rochas, Rinaudo, & Ribeiro-Claro, 2008). Apart from gelling, carrageenan also provides possibilities to modify texturizing and sensorial properties of food products in terms of extending shelf life, viscosity building, stabilizing, emulsifying, controlling the release of flavor compounds, and so on (Shao, Qin, Han, & Sun, 2014).

*Eucheuma* is a group of red seaweed commonly found in Southeast Asia as source of carrageenan (Huang & Yang, 2019). Carrageenans share a disaccharide backbone of alternating 3-linked β-D-galactopyranose (G) and 4-linked α-D-galactopyranose (D) and distinguished themselves according to the position of sulphates (S) and the existence of cyclization of the D units forming an anhydro ring (DA) (Azevedo et al., 2013). KC and IC (G4S-DA and G4S-DA2S, respectively) exhibit different gelling behaviours as a result of selective sulphation amounts and positions. The extraction conditions influence the components of carrageenan, which directly affect their properties as a food additive (Montolalu, Tashiro, Matsukawa, & Ogawa, 2008). For gel-forming agents, the extraction temperature affects the characteristics and properties of the gel (Sinthusantran, Benjakul, Hemar, & Kishimura, 2016) in terms of their molecular mass, molar mass distribution, shape, size, and the system’s viscosity (Webber, de Carvalho, & Barreto, 2012).

Extraction temperature affects not only the structures but also the components in the extracts. Synergistic effect, which derives from the cooperation of different components, has been manifested in the field of expanding the spectrum of rheological properties. The combination of other polysaccharides, proteins, cations, and small molecules gives novel rheological performance of carrageenan (Derkach, Ilyin, Maklakova, Kulichikhin, & Malkin, 2015; Ramírez-Sucre & Vélez-Ruiz, 2013; Yang, Gao, & Yang, 2020). Mixed carrageenans such as the mixture of KC and IC are reported to form a bicontinuous structure, which enrich the rheological performance of carrageenan (Brenner,
2. Materials and methods

2.1. Materials

Dried food grade *Eucheuma spinosum* was bought from Southeast China. The seaweed was then milled to powder with a particle size of less than 1.0 mm for later extraction.

Analytical grade (AR) chemicals and standards, including potassium bromide (KBr), commercial grade KC and IC as well as benzoylated dialysing tube (MWCO 2000) were obtained from Merck (St. Louis, MO, USA). Solvent, such as ethanol was obtained from Macron Fine Chemicals (Center Valley, PA, USA). Deionised water (DI water) was prepared using a Milli-Q purification facility.

2.2. Extraction of polysaccharides from eucheuma powder

Eucheuma powder was first degreased using 50 vol (v/w) of 85% ethanol at room temperature, with stirring, for three days to remove fat, pigments, and other lipophilic impurities since they were reported to affect the physicochemical properties (Le, Maki, Okazaki, Osako, & Takahashi, 2018). The mixture was vacuum filtered. The residues were extracted using hot DI water for 8 h, with refluxing and stirring, at different temperatures: 60, 70, 80, and 90 °C. The extracts were rotary evaporated under a reduced pressure to 20% of the original volume and precipitated with five volumes of ethanol. The precipitated polysaccharides were collected using centrifugation at 4000g for 5 min. The precipitates were then freeze-dried and re-dissolved in DI water dialyzed against DI water using benzoylated dialyzing tube (MWCO 2000). The residue was freeze-dried again as final extracts used to calculate yield.

2.3. General methods

The total sugar content was determined using the phenol-sulphuric acid method, with galactose as a standard (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956). The sulphate content and cations were determined by elemental analysis on an Elementar vario MICRO cube. The molecular weight distribution was detected using aqueous gel permeation chromatography (GPC) on a Waters e2695 Alliance system, with a Waters 2414 RI detector and Styragel HR 3 and Styragel HR 4 columns. A solution of 0.2 mol/L NaCl was prepared as both the sample solvent and the mobile phase, at a flow rate 0.8 mL/min. The column was kept at 30 °C (Hu et al., 2014).

2.4. Characterization of components in the extracts

The FTIR spectra were recorded on a PerkinElmer Spectrum One FTIR spectrometer using the KBr pellet pressing method. Spectra were acquired and then processed using Spectrum software version 6.3.2. The spectra were scanned at room temperature in transmission mode over the wave number range of 4000–500 cm⁻¹ with a scan number of 64 to produce an average spectrum. The background spectrum of air was scanned under the same test conditions before each group of measurements. Triplicates of each sample were tested (Sheng et al., 2018).

¹H NMR spectra and heteronuclear single quantum coherence (HSQC) spectra were obtained using a Bruker AV500 instrument at 25 °C. Forty milligram extracts from different extraction conditions were dissolved in 0.5 mL D₂O (99.9%) and determined separately.

2.5. Atomic force microscopy (AFM)

The polysaccharide samples were dissolved in DI water with mechanical agitation at room temperature at a concentration of 2 mg/mL.
as a stock solution. They were then diluted with DI water to final concentrations of 0.02 mg/mL and 0.002 mg/mL. Test solutions (10 μL) of each sample were deposited onto freshly cleaved mica and blown gently to form an even, thin film. AFM images were acquired in Tapping Mode™ on a Multimode™ atomic force microscope (Veeco, Santa Barbara, CA, USA) connected to a Nanoscope V™ controller. The resonance frequency was 160–225 kHz, the force constant was 25–90 N/m and the scan speed was 0.4 Hz. All polysaccharide sample determinations were carried out in air at room temperature.

Fig. 2. Nuclear magnetic resonance (NMR) spectra of polysaccharide extracts: $^1$H NMR spectrum of: (a). 60 °C extract; (b). 70 °C extract; (c). 80 °C extract; (d). 90 °C extract; Heteronuclear single quantum coherence (HSQC) spectrum: (e). 60 °C extract; (f). 70 °C extract; (g). 80 °C extract; (h) 90 °C extract.
2.6. Sample preparation for rheological test

The samples were all tested as 2 g/100 mL homogenous solution dissolved in DI water under mild magnetic stirring at 90 °C for 1 h. To investigate the synergistic effect, commercial KC ratio was set to be 0, 20%, 40%, 60%, 80% and 100% separately. Commercial IC was also mixed with KC to obtain reference samples.

2.7. Oscillatory rheological test

The rheological measurements were conducted in a rotational Anton Paar MCR 102 stress-controlled rheometer (Anton Paar, Graz, Austria) equipped with a Peltier temperature controller. A stainless-steel cone and plate geometry (angle: 1 and diameter: 60 mm) was used. Before measurements, preheated sample solution was poured onto the plate and covered with sunflower oil on the edge of the sample to avoid evaporation. Dynamic oscillatory or viscoelastic measurements were performed to assess the gelation kinetics and properties as shown below. (1) Temperature sweep (1 °C/min): temperature decreased from 90 °C to 20 °C. (2) Time sweep (1 point/min); at 20 °C for 3 h. During these two procedures, angular frequency was set to be constant as 1 rad/s with a constant strain 1% to monitor gelation evolution. (3) Frequency sweep: angular frequency from 100 to 1 rad/s with a fixed strain 1%. Power law was used to interpret the frequency sweep result in terms of complex viscosity \( \eta^* \):

\[
\eta^* = A\omega^n
\]

where \( A \) is reported to be related to gel strength, \( \omega \) represents angular frequency (1/s) and \( n \) as relaxation exponent indicates the correlation in the gel network. (Gabriele, de Cindio, & D’Antona, 2001). (4) Strain amplitude sweep: strain from 0.1% to 10,000% with a fixed angular frequency of 1 rad/s to characterize the large deformation rheological properties. The \( G' \) (storage modulus) and \( G'' \) (loss modulus) were recorded during the above dynamic rheological measurements (Alamprese & Mariotti, 2011; Bi, Li, Wang, & Adhikari, 2017) Cohesive energy density \( E_c \) can be calculated using Equation (2) according to strain sweep curves and the deformation behavior:

\[
E_c = \int_0^\gamma \sigma \gamma \frac{G''}{G'} \, d\gamma = \frac{1}{2} \gamma_c \sigma \frac{G''}{G'}
\]

where \( \gamma_c \) is the critical strain (strain when \( G'' \) becomes higher than \( G' \)), \( G' \) is the corresponding storage modulus.

Table 2

Effects of extraction temperature on the polysaccharide dimensions characterized by AFM images.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Height (nm)</th>
<th>Step Height (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>0.57 ± 0.17ᵃ</td>
<td>0.76 ± 0.27ᵃ</td>
</tr>
<tr>
<td>70</td>
<td>0.50 ± 0.13ᵇ</td>
<td>0.61 ± 0.29ᵇ</td>
</tr>
<tr>
<td>80</td>
<td>0.49 ± 0.09ᵇ</td>
<td>0.60 ± 0.27ᵇ</td>
</tr>
<tr>
<td>90</td>
<td>0.48 ± 0.10ᵇ</td>
<td>0.58 ± 0.25ᵇ</td>
</tr>
</tbody>
</table>

Results are the mean ± standard deviation. One-way ANOVA: different letters indicate significant differences among the different extracts (\( P < 0.05 \)).

Table 3

Composition and molecular weight analysis of carrageenan standards and polysaccharides from Eucheuma.

<table>
<thead>
<tr>
<th>Hydrocolloid source</th>
<th>KC</th>
<th>IC</th>
<th>Eucheuma</th>
<th>Eucheuma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C g/100 g</td>
<td>28.70 ± 0.15ᵃ</td>
<td>22.44 ± 0.08ᵇ</td>
<td>21.30 ± 1.04ᵇ</td>
<td>23.96 ± 1.01ᵇ</td>
</tr>
<tr>
<td>H g/100 g</td>
<td>5.19 ± 0.67ᵃ</td>
<td>4.19 ± 0.48ᵇ</td>
<td>3.94 ± 0.21ᵇ</td>
<td>4.19 ± 0.21ᵇ</td>
</tr>
<tr>
<td>S g/100 g</td>
<td>5.38 ± 0.19ᵇ</td>
<td>9.20 ± 0.72ᵃ</td>
<td>9.62 ± 0.54ᵃ</td>
<td>9.43 ± 0.50ᵇ</td>
</tr>
<tr>
<td>Mw (Da)</td>
<td>48,913</td>
<td>55,843</td>
<td>47,890</td>
<td>40,623</td>
</tr>
<tr>
<td>Mn (Da)</td>
<td>24,231</td>
<td>27,246</td>
<td>11,250</td>
<td>10,348</td>
</tr>
<tr>
<td>D m = Mw/Mn</td>
<td>2.02</td>
<td>2.05</td>
<td>4.26</td>
<td>3.93</td>
</tr>
</tbody>
</table>

Results are the mean ± standard deviation. One-way ANOVA: different letters indicate significant differences among the different polysaccharides (\( P < 0.05 \)).
2.8. Statistical analysis

All experiments were performed in triplicate, and data are expressed as the mean ± standard deviation. Analysis of variance (ANOVA) was used to evaluate the significant differences among various treatments (using the SPSS software 17.0, SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant if $P < 0.05$.

3. Results and discussion

3.1. Compositional analysis of eucheuma extracts

The yield (30.33–24.49%) is reasonable as compared to previous research (Souza et al., 2012) as shown in Table 1. The other components, such as total sugar content and ion contents, are also shown. The ion contents did not show obvious variation with temperature, which made the comparison among different extracts acceptable, because the ions can significantly affect the behavior of carrageenan (Nickerson, Darvesh, & Paulson, 2010). Total sugar contents of 70 and 80 °C extracts were lower than 60 and 90 °C extracts.

3.2. Component characterization using FTIR

The composition of the polysaccharide extract was further determined using FTIR, as shown in Fig. 1. The absorption around 1250 cm$^{-1}$ confirmed the existence of sulphate groups, which were assigned to the asymmetric stretching vibration of $\text{O=S=O}$ (Pereira et al., 2009). Absorptions at 840 cm$^{-1}$, indicated that the sulphate ester was mainly at the C-4 for IC (Bilan, Grachev, Shashkov, Nifantiev, & Usov, 2006). The absorption at 805 cm$^{-1}$ was said to come from...
sulphate ester in the 2-position of the anhydro-D-galactose residues (DA2S), which is a characteristic signal of IC (Pereira et al., 2009). The results agreed with the previous studied that red seaweed can be a source of IC (Diharmi, Fardiaz, Andarwulan, & Heruwati, 2017).

3.3. Component characterization using NMR

$^1$H NMR spectra provided more structural information as shown in Fig. 2. $^1$H NMR could be used to determine the existence of different carrageenan repeating units. The peaks at 5.29 ppm were from anomeric protons of KC. For the carrageenan precursors containing $\nu$ carrageenan and $\mu$ carrageenan, their unique chemical shifts were at 5.50 ppm and 5.24 ppm, respectively (van de Velde et al., 2005).

The HSQC spectra shown in Fig. 2 also indicated a similar composition. Signals at the anomeric region confirmed the existence of $\iota$ carrageenan. A2S, a unit found mainly in KC carrabiose, showed its chemical shift at C1/H1: 92.8/5.33 ppm (Kumar, Godiya, & Siddhanta, 2012). For the signal found at C1/H1: 5.27/100.4 ppm, it may be derived from the existence of reserve $\alpha$-glucans as the extraction temperature increased (Perez Recalde et al., 2016). According to the NMR results, the carrageenan in Eucheuma was most likely to be $\iota$ carrageenan.

3.4. AFM images of eucheuma extracts

The AFM images of the extracts are shown in Fig. 3. A previous study reported many bright parts at higher concentrations that were caused by the strong self-aggregation of carrageenan (Sow, Kong, & Yang, 2018) and the black areas caused by the water evaporation from liquid samples (Ortiz-Tafoya, Rolland-Sabaté, Garnier, Valadez-Garcia, & Tecante, 2018). To further explore the morphology difference, several parameters are chosen to explain the AFM images as shown in Table 2.

The height of the polysaccharide molecule could represent the degree of molecular association. There are two types of intermolecular association commonly found in food polysaccharides: side-by-side association and end-to-end association. Side-by-side association contributes more to the vertical height of a polysaccharide (Funami, 2010). In this study, there was a decrease in the heights of the polysaccharides in the extracts as the temperature increased. Lower temperatures tended to result in polysaccharides with side-by-side association. Side-by-side association happens mostly at the presence of 3, 6-anhydrgalactose, causing branching and higher heterogeneity of the network (Morris et al., 2001).

3.5. Comparison of eucheuma extracts and commercial carrageenan

According to the previous results, 60 and 80 °C extracts were selected to represent mild extraction and high temperature extraction considering yields, sugar contents and microstructure. The comparison between Eucheuma extracts and commercial carrageenan are listed in Table 3. Eucheuma extracts have comparable sulphated esters as IC. Accordingly, the percentage of carbon and hydrogen are less than commercial KC. The average molecular weights of the samples are around 50,000 Da, which is in agreement of the previous research (Diharmi et al., 2017) and both the number average molecular weight (Mn) and the weight average molecular weight (Mw) of extracts are lower than commercial values. Molar mass dispersity (Đm), a parameter describing the uniformity and dispersity of polymers, exhibits that both of the carrageenan standards (Đm = 2) are linear polymers with high degree of polymerisation. For the extracts, heterogeneity was found and lower temperature one acquired even higher heterogeneity with higher...
### Table 4

<table>
<thead>
<tr>
<th>Composition</th>
<th>Gelation</th>
<th>Power law fitting</th>
<th>Strain sweep calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gelling temperature (°C)</td>
<td>Gel strength (G′ at 20 °C, Pa)</td>
<td>A(10^9 Pa s^n), n</td>
</tr>
<tr>
<td>80 g/100 g K</td>
<td>34.85 ± 0.27 ^c</td>
<td>98.33 ± 0.98</td>
<td>94.33 ± 0.78</td>
</tr>
<tr>
<td>60 g/100 g K</td>
<td>41.87 ± 0.27 ^b</td>
<td>94.00 ± 0.00</td>
<td>94.00 ± 0.00</td>
</tr>
<tr>
<td>20 g/100 g K</td>
<td>53.34 ± 0.27 ^a</td>
<td>95.00 ± 0.00</td>
<td>95.00 ± 0.00</td>
</tr>
</tbody>
</table>

*Within each column, means with different lowercase letters are significantly different (P < 0.05) among different groups.*

### 3.6. Gelling ability of eucheuma extracts

All the samples were tested in the concentration of 2% (w/v) in Milli-Q water. KC usually starts the rapid increase earlier in the gelling process (Rosal et al., 2017). For IC-based *Eucheuma* extracts, they start sol-gel transition at a relatively lower temperature than *κ* carrageenan standards as shown in Fig. 4a. Meanwhile, the gel strengths, in terms of *G*′ value at 25 °C (Das, Choudhary, & Thompson-Witrick, 2019), of *Eucheuma* extracts are relatively lower than the commercial *κ* carrageenan samples. Gelling temperature of *Eucheuma* extracts exhibits a tendency of melting for the lower gelling points. Moreover, the gel strength of 80 °C extracts is slightly greater than 60 °C extracts.

### 3.7. Mechanical properties of eucheuma extracts

Frequency sweep results are shown in Fig. 4b, c, 4d and 4e. *G*′ (elastic rigidity) seems to be stable in KC and IC while for *Eucheuma* extracts, *G*′ increased a bit as angular frequency went up. Complex viscosity *η*″ is also recorded along with frequency sweep. Viscosity participates in the texture and mouthfeel of the products and high viscosity help decrease the grittiness (Amador, Hartel, & Rankin, 2017). Therefore, *Eucheuma* extracts give less thickening and smooth mouthfeel. As for strain sweep, not only the LVR is provided, it also notes the deformation strain of the gels. From the results, *Eucheuma* extracts are more difficult to deform than commercial standards.

### 3.8. Synergistic effects with KC

KC mixed with IC, 60 °C extracts and 80 °C extracts are tested in different ratio (80% KC, 60% KC, 40% KC and 20% KC, w/w) and the cooling curves are shown in Fig. 5. KC/IC mixtures tend to undergo two-step gelation, which is the pattern found in previous research (Bai, Nguyen, Renou, & Nicolai, 2019). The two steps come from two independent networks forming of KC and IC separately. However, when it comes to *Eucheuma* extracts, whose major component is also IC, there is no obvious second step of gelation. The discrepancy may be owing to the weak gelling property of extracts as shown in the previous cooling tests.

Though undergoing different gelling procedures due to lower individual gelation ability, the gel strength seems to suggest a similar synergistic effect among all IC-based samples as shown in Table 4. Synergistic effect is defined as the overall effect greater than the sum of individual components (Schiassi et al., 2019). This is found in 80%, 60% and 40% KC mixtures in Fig. 5a, b and 5c. Moreover, the synergistic effect seems stronger in 60% and 40% KC mixtures of 80 °C extracts and 60% KC mixture of 60 °C extracts. For 20% KC, gelation mainly comes from IC-based components. Due to their weaker gelling ability, the synergistic effect is inconspicuous. The value of A from power law model fitting aligns with the findings of gel strength.

Though working a lot in the performance of gel strength, *Eucheuma* extracts contribute less to the maintenance of gelling temperature as listed in Table 4. Commercial IC complete gelation before KC while *Eucheuma* extracts fail to gelling themselves because of lower gelling ability. Therefore, as the ratio of KC (major gelling agent) decreases, the effective gelling concentration decreases and provides lower gelling temperature (Wang, Tong, Luo, Xu, & Ren, 2016).

Frequency sweep results are shown in Fig. 6. From the figure, a growing trend of frequency dependence was found as the decrease of KC ratio. This dependency is reported to be the result of the structural rearrangement and relaxation processes in the gel caused by high shear frequency (Diáz et al., 2019). For these typical gels (40%, 60% and 80% KC), *Eucheuma* extracts behave like IC and even provide slightly stronger gels with higher *G*′ value. From power law model fitting, lower relaxation exponent *n* indicates that 20% KC mixture has lower...
elasticity and higher viscosity (Sow, Nicole Chong, Liao, & Yang, 2018). For strain sweep, IC has greater fracture strain. Therefore, the addition of IC enlarged the deformation strain as shown in Fig. 7. Comparing to commercial IC, 60 °C Eucheuma extracts contributed less to the extension of breaking strain. As for cohesive energy density (Ec), illustrating a decreasing linear relationship with crosslink density (Li & Strachan, 2018), it shows a decrease in crosslink density: IC < 80 °C extract < 60 °C extract with KC. To sum up, Eucheuma extracts delivered comparable contribution to the gel strength while produced less mechanical improvements than IC. This may result from the heterogeneity and the lower molecular weight and offer diversity of mouthfeel.

3.9. Schematic model for the synergistic effect

KC and IC shows theoretical synergistic effect coming from the individual gels formed by them. As for Eucheuma extracts, whose major component is IC with lower molecular weight, the synergistic effect is also found even though their individual gel-forming ability is rather low. Due to their heterogeneity and lower molecular weight, it seems they participate in the synergistic effect mainly by acting as density-building components. In other words, the extracts fill in the 3D network produced by carrageenan and increase the density of the structure. As a result, the gel strength got raised. The variation exists in the composition of the extracts. Low temperature extract has greater heterogeneity comparing to high temperature extract while they can both facilitate the gel strength even though they can hardly form gels themselves. Accordingly, low temperature extract forms more crosslink in the mixed gel as indicated by rheological test. Therefore, high temperature extract supplies better synergistic effect in terms of gel strength and the schematic presentation is shown in Fig. 8.

4. Conclusion

This study presents the rheological properties of the polysaccharide extracts from Eucheuma and the synergistic effect of kappa carrageenan addition in them. It was found that the gelling ability varies among carrageenan and Eucheuma extracts (kappa carrageenan > iota carrageenan > Eucheuma extracts). Moreover, when mixed together in the ratio of 80% KC, Eucheuma extracts deliver stronger gelling ability than Kappa carrageenan at 60°C, and the ratio of 60% KC, stronger gelling ability can be found in the Eucheuma extracts. These results provide reference for the seaweed-based beverage design (with hot water extraction) and production and allow the possibility to improve the viscoelastic property with kappa carrageenan of the products. Comparable amounts of KC and Eucheuma extracts are more suitable for the application of stronger gels. However, to better understand the interaction in the mixed system, more investigation is required to elucidate the structural changes. Further research about the beverage system also needs to be considered since diverse food biopolymers, small molecules as well as

Fig. 6. Storage modulus $G'$ and loss modulus $G''$ during frequency sweep: (a) 80% (w/w) KC; (b) 60% (w/w) KC; (c) 40% (w/w) KC and (d) 20% (w/w) KC mixed with IC, 60 °C extract and 80 °C extract. (Solid symbol: $G'$; empty symbol: $G''$; $\times$ IC; $\bigodot$ 60 °C extract; $\bigotimes$ 80 °C extract).
ions contribute to the rheological behaviors, too.

CRediT authorship contribution statement

Dongying Yang: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing - original draft. Hongshun Yang: Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing.

Declaration of competing interest

We declare that we have no commercial or associative interest that represents a conflict of interest in connection with this manuscript. We have no financial and personal relationships with other people or organisations that can inappropriately influence our work.

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References


