CHARACTERIZATION OF KAPPA-CARAGEENAN FROM THE RED ALGA KAPPAPHYCUS STRIATUM

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SUMMARY

The red alga Kappaphycus striatum is an economically important species and extensively cultivated in Vietnam as a material source for carrageenan production. To evaluate carrageenan quality, the characterization of carrageenan extracted from this alga was investigated. As a result, chemical composition of carrageenan consists of 32.4% of 3,6 anhydrogalactose and 24.3% of sulfate. Gelling and melting temperatures are 34.4°C and 55.6°C, respectively. Gel strength of 1.5% is 615 g/cm² and average molecular weight is about 267 kDa. Furthermore, FT-IR spectrum showed intense absorption bands at 930 cm⁻¹ and 850 cm⁻¹ that attributed to 1,4-linked 3,6 anhydro-α-D-galactose and 1,3-linked β-D-galactose-4-sulfate of kappa-carrageenan, respectively. 13C NMR spectrum indicated the signals for anomeric carbon of β-D-galactose-4-sulfate at 102.6 ppm and anomeric carbon of 3,6-anhydro-α-D-galactose at 95.3 ppm. 1H NMR spectrum showed peak signals at 3.57 ppm and 5.1 ppm that corresponds with O-methyl proton of 1,3-linked 6-O-methyl-D-galactose and α-anomeric proton of 3,6 anhydro-α-D-galactose residues, respectively. The results show that the carrageenan from the red alga Kappaphycus striatus is kappa-carrageenan with the repeating disaccharide unit consisting of 1,3-linked 6-O-methylated, β-D-galactose-4-sulfate and 1,4-linked 3,6 anhydro-α-D-galactose and did not contain iota-carrageenan. Therefore, this alga may promise to be a good source for carrageenan production for application in food or medicine.

Keywords: Carrageemophytes, kappa-carrageenan, Kappaphycus striatum, properties, structure

INTRODUCTION

Carrageenan is extracted from red seaweed of the Rhodophycea family commonly from genera such as Eucheuma, Solieria, Cripus, Agardhiella, Chondrus, Hypnea, Sarconema and Iridaea (Zia et al., 2017). Eucheuma and Kappaphycus seaweeds are most commonly cultivated seaweed across Malaysia and Southeast Asia (Hurtado et al., 2014; Zuldin et al., 2016).

Carrageenan is the general name for a group of high molecular weight sulphated polysaccharides obtained by alternate units of D-galactose and 3,6-anhydro-galactose joined by -1,3 and -1,4-glycosidic linkage (Figure 1) (Craigie, 1990). There are three main types of carrageenan, which vary in their degree of sulfation (Prajapati et al., 2014; Li et al., 2014; Liu et al., 2015). Kappa-carrageenan is composed of alternating 3-linked β-D-galactose-4-sulfate and 4-linked 3,6-anhydro-α-D-galactopyranose having one sulfate group per disaccharide repeating unit. Iota-carrageenan is composed of alternating 3-linked β-D-galactose-4-sulfate and 4-linked 3,6-anhydro-α-D-
galactose-2-sulfate having two sulfate groups per disaccharide repeating unit. Lambda-carrageenan has three sulfate groups per disaccharide unit but do not exhibit any 3,6-anhydride bridges contrary to kappa- and iota-carrageenan (Palvi et al., 2011). The main differences which effect on the properties of different carrageenans are the number and position of ester sulfate groups and the content of 3,6-anhydro-galactose. Higher levels of ester sulfate resulted in lower gel strength and solubility temperature (Necas, Bartosikova, 2013).

![Figure 1. Idealized repeating structure of carrageenan.](image)

Carrageenans possess various pharmaceutical properties including immunomodulatory, antitumor, anticoagulant activities and anti-hyperlipidemic (Prajapati et al., 2014). Recently, they have been used in controlled drug release delivery systems (Liang et al., 2014; Zia et al., 2017; Yegappan et al., 2018), as well as enhancing dissolution rates of poorly soluble substances.

The red algae, carrageenophytes, *Kappaphycys alvarezi*, *Kappaphycus striatum* and *Eucheuma denticulatum* are economically important food species and extensively cultivated in Vietnam as a source of not only carrageenan, but also as a source of bioactive compounds for biochemical and medicinal application (Le Dinh Hung et al., 2009, 2011, 2015, 2019). However, little information is known about structures of carrageenans from these algae, except for the structure of carrageenan from *E. denticulatum* (Thanh Thi Thu Thuy et al., 2007). Thus, the objective of the present research was to elucidate the properties and structure of kappa-carrageenan from the red alga *K. striatum* cultivated at Vanphong Bay, Khanhhoa province, Vietnam, which will provide more valuable information of carrageenan group from these algae for applications in food or medicine.

**MATERIALS AND METHODS**

The red alga *K. striatum* (F. Schmitz) Doty ex Silva was collected at Vanphong Bay, Khanhhoa province (120°29’ N, 109°10’ E), Vietnam in March, 2019, brought to the laboratory, and kept at -20°C until use. Rhodizolate natri, acetal, resorcinol, D-fructose was obtained from Merck (Germany).

**Extraction of carrageenan**

Carrageenan was extracted according to the method of Ohno et al. (1994). Dry algal powder was treated in 6% KOH at 80°C for 2 h. Then, the algal powders were collected, washed by distilled water to remove excess alkali and extracted in distilled water at 90°C for 2 h. Thereafter, the solution was filtered and the extract was gellified with 0.2% KCl, frozen and thawed at least twice with distilled water to recover carrageenan, dried at 60°C and ground into power.

Prior to determine chemical and structural characterization, carrageenan sample was repurified by dialysis using a Spectrapor Membrane with Mw cut-off 12,000–14,000 Da, against milli-Q water for 24 h with frequent changes of water. After dialysis,
the sample was filtrated by filter (0.45 μm) and precipitated by absolute ethanol, dried at 40°C and milled into powder.

**Determine the 3,6-anhydrogalactose content**

3,6-anhydrogalactose content was determined according to the method of Yaphe, Arsenault (1965), using D-fructose as a standard. Briefly, 2 mL of a solution containing up to 0.25 μM of fructose or 3,6-anhydrogalactose (polysaccharide about 100 – 120 μg/mL) was transferred to a boiling tube and covered with a glass marble. The tubes were placed in an ice bath and cooled for at least 3 min. The tubes were allowed to stand 10 min in the dark and were then heated for 10 min at 80°C. It was then cooled for 1.5 min in an ice bath and the absorbance was measured within 15 min at 555 nm. 3,6-anhydrogalactose content was determined by multiplying to value of 1.087. The assay was carried out in triplicate for each test solution.

**Determine the sulfate content**

Sulfate contents were determined according to the method of Terho, Hartiala (1972), using Na₂SO₄ as a standard. Briefly, 0.5 mL each sample (polysaccharide about 100 – 120 μg/mL), standards and water are pipetted into test tubes and 2.0 mL ethanol is added to each tube. 1.0 mL BaCl₂ buffer (10 mL of acetic acid 2 M, 2 mL of BaCl₂ 0.005 M, 8 mL of NaHCO₃ 0.02M and 80 mL ethanol) and 1.5 mL sodium rhodizonare solution (5 mg of rhodizonate natri dissolved in 20 mL water and 80 mL ethanol) were added to each tube and shaken well. The tubes were allowed to stand 10 min in the dark at room temperature and measured at 520 nm in 30 min. The assay was carried out in triplicate for each test solution.

**Determine gelling and melting temperatures of κ-carrageenan**

Gelling and melting temperatures were determined according to the method of Hellebust, Craig (1978). Gelling temperature was measured with 1.5% kappa-carrageenan solution in 0.2% KCl by a thermometer, corresponded to the introduced glass beads (diam: 4.30 mm; wt: 430 mg) which failed to sink to the bottom of the test tube at an interval of 0.5°C. The melting temperature corresponded to the temperature at which glass beads (diam: 4.30 mm; wt: 430 mg) gradually sank to the bottom of the test tube; temperature gradually was raised at an interval of 0.5°C. The assay was carried out in triplicate for each test solution.

**Determine the viscosity-average molecular weight and gel strength**

The viscosity-average molecular weight (MW) was obtained from viscometry and extrapolation. The sample was dissolved at initial concentration of 0.1% in 0.1 M NaCl. Viscosity measurements at different diluted concentrations of kappa-carrageenan in 0.1 M NaCl were done at 25 ± 0.1°C from efflux time of polymer solution (t) and that of solvent 0.1 M NaCl (t₀), relative viscosity rel = t/t₀ was obtained. Specific viscosity was calculated from the relationship sp = rel - 1. Reduced viscosity for a set of polymer solutions was calculated at different concentrations (g/mL). Intrinsic viscosity was then obtained from common ordinate intercept on extrapolation of plots of reduced viscosity versus concentration (Pal *et al.*, 2008). The Mw was calculated according to the Mark–Houwink equation for kappa-carrageenan in 0.1 M NaCl at 25°C.

\[
[\eta] = K \cdot M_w^\alpha
\]

\[K = 8.84 \times 10^{-3}\] and \[\alpha = 0.86\], according to the report of Vreeman *et al.* (1980).

Gel strength was measured on a Rheometer (CR-500DX; Sun Scientific, Tokyo, Japan), using 1.5% kappa-carrageenan solution in 0.2% KCl. The assay was carried out in triplicate for test solution.

**Determine structures**

Fourier-Transform Infrared (FT-IR)
The infrared spectrum of kappa-carrageenan was shown in Figure 2. As reported previously, the bands at 1260 cm\(^{-1}\), 850 cm\(^{-1}\) were assigned respectively to \(\text{O} = \text{S} = \text{O}\) symmetric vibration and \(\text{C}_4\text{O} - \text{S}\) stretching vibration, respectively. They stood for total \(-\text{SO}_4\) and \(\text{C}_4\text{O} - \text{S}\) of \(\beta\)-D-galactose, respectively. The band at 930 cm\(^{-1}\) proved the existence of \(\text{C} = \text{O} - \text{C}\) of 3,6-anhydro-

![FT-IR spectrum of carrageenan extracted from K. striatum.](image)

**Figure 2.** FT-IR spectrum of carrageenan extracted from *K. striatum.*

The infrared spectrum of kappa-carrageenan was shown in Figure 2. As reported previously, the bands at 1260 cm\(^{-1}\), 850 cm\(^{-1}\) were assigned respectively to \(\text{O} = \text{S} = \text{O}\) symmetric vibration and \(\text{C}_4\text{O} - \text{S}\) stretching vibration, respectively. They stood for total \(-\text{SO}_4\) and \(\text{C}_4\text{O} - \text{S}\) of \(\beta\)-D-galactose, respectively. The band at 930 cm\(^{-1}\) proved the existence of \(\text{C} = \text{O} - \text{C}\) of 3,6-anhydro-

### Table 1. Characterization of kappa-carrageenan from *K. striatum.*

<table>
<thead>
<tr>
<th>Carrageenan yield (% dry alga)</th>
<th>3,6-AG content(^a) (% carrageenan)</th>
<th>Sulfate content (% carrageenan)</th>
<th>Gelling temp (°C)</th>
<th>Melting temp (°C)</th>
<th>Gel strength (g/cm(^2))</th>
<th>MW(^b) (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>39.2 ± 3.5</td>
<td>32.4 ± 0.5</td>
<td>24.3 ± 0.8</td>
<td>34.4 ± 0.9</td>
<td>55.6 ± 1.6</td>
<td>615 ± 45</td>
<td>267</td>
</tr>
</tbody>
</table>

\(^a\) AG: anhydrogalactose; \(^b\) MW: viscosity-average molecular weight. Mean ± SEM (n = 3).
D-galactose (Silva et al., 2010). It also displayed an absorbance band at 1159 cm\(^{-1}\) due to bridge \(-\)O stretch and band at 1070 cm\(^{-1}\) was reported to be related to C–O stretch (Mendoza et al., 2002; Tranquilan-Aranilla et al., 2012). The native carrageenan extracts were composed predominantly of kappa carrageenan with trace amounts of iota-carrageenan that showed at absorption band of 805 cm\(^{-1}\) for 3,6 anhydro-\(\alpha\)-D-galactose-2-sulfate (Mendoza et al., 2002). However, the infrared spectrum in this study showed no appearance of absorption band at 805 cm\(^{-1}\), indicating that kappa-carrageenan sample did not contain iota-carrageenan.

![Figure 3](image)

**Figure 3.** \(^{13}\)C NMR spectrum of carrageenan extracted from *K. striatum*.

**Table 2.** Chemical shifts (ppm) in the \(^{13}\)C NMR spectrum of kappa-carrageenan extracted from *K. striatum*

<table>
<thead>
<tr>
<th>Unit</th>
<th>Carbon atom</th>
<th>Kappa-carrageenan</th>
<th>Ref(^a)</th>
<th>Ref(^b)</th>
<th>Ref(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Galactose-4-sulfate</td>
<td>C-1</td>
<td>102.6</td>
<td>102.6</td>
<td>102.7</td>
<td>102.9</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>69.7</td>
<td>69.6</td>
<td>69.9</td>
<td>70.1</td>
</tr>
<tr>
<td></td>
<td>C-3</td>
<td>78.7</td>
<td>78.9</td>
<td>79.1</td>
<td>79.5</td>
</tr>
<tr>
<td></td>
<td>C-4</td>
<td>74.1</td>
<td>74.1</td>
<td>74.3</td>
<td>74.5</td>
</tr>
<tr>
<td></td>
<td>C-5</td>
<td>74.7</td>
<td>74.7</td>
<td>75.0</td>
<td>75.2</td>
</tr>
<tr>
<td></td>
<td>C-6</td>
<td>61.4</td>
<td>61.2</td>
<td>61.5</td>
<td>61.7</td>
</tr>
<tr>
<td>3,6-anhydro-D-galactose</td>
<td>C-1</td>
<td>95.3</td>
<td>95.2</td>
<td>95.4</td>
<td>95.7</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>69.9</td>
<td>69.9</td>
<td>70.1</td>
<td>70.4</td>
</tr>
<tr>
<td></td>
<td>C-3</td>
<td>78.9</td>
<td>79.1</td>
<td>79.4</td>
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<td>C-4</td>
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<td>78.7</td>
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<td>C-5</td>
<td>76.7</td>
<td>76.7</td>
<td>77.0</td>
<td>77.2</td>
</tr>
<tr>
<td></td>
<td>C-6</td>
<td>69.4</td>
<td>69.4</td>
<td>69.7</td>
<td>69.9</td>
</tr>
</tbody>
</table>

\(\text{C1–6 shows the carbon numberings.}\)

\(^{a,b,c}\) Referenced to Mendoza et al. (2002), Kolender, Matulewicz (2004) and Tranquilan-Aranilla et al. (2012), respectively.
Figure 4. $^1$H NMR spectrum of carrageenan extracted from *K. striatum*.

Table 3. Chemical shifts (ppm) in the $^1$H NMR spectrum of carrageenan extracted from *K. striatum*.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Proton</th>
<th>Kappa-carrageenan</th>
<th>Ref $^a$</th>
<th>Ref $^b$</th>
<th>Ref $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Galactose-4-sulfate (G4S)</td>
<td>H-1</td>
<td>4.64</td>
<td>4.75</td>
<td>4.75</td>
<td>5.19</td>
</tr>
<tr>
<td></td>
<td>H-2</td>
<td>3.60</td>
<td>3.74</td>
<td>3.50</td>
<td>3.67</td>
</tr>
<tr>
<td></td>
<td>H-3</td>
<td>3.98</td>
<td>4.10</td>
<td>3.90</td>
<td>3.96</td>
</tr>
<tr>
<td></td>
<td>H-4</td>
<td>4.83</td>
<td>4.95</td>
<td>4.83</td>
<td>4.84</td>
</tr>
<tr>
<td></td>
<td>H-5</td>
<td>3.80</td>
<td>3.93</td>
<td>3.71</td>
<td>3.72</td>
</tr>
<tr>
<td></td>
<td>H-6</td>
<td>3.97</td>
<td>3.93</td>
<td>3.71</td>
<td>3.71</td>
</tr>
<tr>
<td>3,6-anhydro-D-galactose (DA)</td>
<td>H-1</td>
<td>5.10</td>
<td>5.24</td>
<td>5.01</td>
<td>5.01</td>
</tr>
<tr>
<td></td>
<td>H-2</td>
<td>4.14</td>
<td>4.12</td>
<td>4.05</td>
<td>4.01</td>
</tr>
<tr>
<td></td>
<td>H-3</td>
<td>4.51</td>
<td>4.30</td>
<td>4.44</td>
<td>4.45</td>
</tr>
<tr>
<td></td>
<td>H-4</td>
<td>4.52</td>
<td>4.75</td>
<td>4.51</td>
<td>4.52</td>
</tr>
<tr>
<td></td>
<td>H-5</td>
<td>4.61</td>
<td>4.65</td>
<td>4.56</td>
<td>4.58</td>
</tr>
<tr>
<td></td>
<td>H-6</td>
<td>4.31</td>
<td>4.25</td>
<td>4.23</td>
<td>4.13</td>
</tr>
</tbody>
</table>

H1–6 shows the proton numberings

$^a$, $^b$, $^c$ Referenced to Campo *et al.* (2009), Abad *et al.* (2011) and Mahmood *et al.* (2014), respectively.

Figure 3 showed the chemical shifts in the $^{13}$C NMR spectrum of kappa-carrageenan that corresponded strong intensity signals of the twelve carbon atoms in the disaccharide repeating unit indicated in Table 2, and agreed closely with reported values (Mendoza *et al.*, 2002; Tranquilan-Aranilla *et al.*, 2012). On the other hand, the signals at 102.6 ppm and 95.3 ppm of kappa-carrageenan spectra have been reported for anomeric carbon of D-galactose-4-sulfate and 3,6-anhydro-D-galactose, respectively (Mendoza *et al.*, 2002). A weak signal at 59.2 ppm is clearly seen in the spectra, which corresponds to the methoxyl group on C-6 of the β-D-galactose-4-sulfate units reported (Mendoza *et al.*, 2002). Figure 4 showed the chemical shifts in the $^1$H NMR spectrum of kappa-carrageenan that corresponded strong intensity signals of the protons in the disaccharide repeating unit indicated in Table 3 and agreed closely with reported values (Campo *et al.*, 2009; Abad *et al.*, 2011; Mahmood *et al.*, 2014).
On the other hand, the signal at 3.57 ppm corresponds to O-methyl proton of 3-linked 6-O-methyl-D-galactose in kappa-carrageenan, indicated methylation in the C-6 position of D-Galactose-4-sulfate. Similar chemical shift (3.56 ppm, 3.58 ppm and 3.58 ppm) for such O-methyl proton was also reported for 6-O-methyl-D-galactose residue (Abad et al., 2011; Mahmood et al., 2014), respectively.

Small variations of the chemical shift values in NMR spectra may be due to the samples collected from different sources or experiments were performed in different conditions (Velde et al., 2004).

The result showed that structure of carrageenan from K. striatum consists of repeating disaccharide unit of β-D-galactose-4-sulfate and 3,6 anhydro-α-D-galactose (Figure 4). Similar structures have been reported for carrageenans from the red algae Eucheuma denticulatum (Thanh Thi Thu Thuy et al., 2007) and E. gelatinae (Tran Dinh Toai et al., 2004), except for difference in structure of iota-carrageenan from Eucheuma had sulfate group at position C2 of 3,6-anhydro-α-D-galactose residue.

CONCLUSION

Carrageenan from the red seaweed of K. striatum is kappa-carrageenan with the repeating disaccharide unit consisting of 1,3-linked 6-O-methylated, β-D-galactose-4-sulfate and 1,4-linked 3,6 anhydro-α-D-galactose. The chemical and structural characteristics of kappa-carrageenan used in this study were similar well to those of the red algae reported, indicating that the red alga K. striatum may promise to be a good source for carrageenan production to apply in food or medicine.

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MÔ TẢ ĐẶC TÍNH CỦA KAPPA-CARRAGEENAN TỪ RONG ĐƠ KAPPAPHYCUS STRIATUM

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TÔM TẮT

Rong đờ Kappaphycus striatum là loại rong kinh tế dạng được nuôi trong rong rải ở Việt Nam làm nguyên liệu để sản xuất carrageenan. Để đánh giá chất lượng carrageenan, đặc tính của carrageenan được chiết từ mẫu rong này đã được khảo sát. Kết quả cho thấy rằng thành phần hóa học của carrageenan bao gồm 32,4% 3,6-anhydrogalactose và 24,3% sulfate. Nhiệt độ tan là 34,4°C và 55,6°C, tương ứng. Độ bền gel 1,5% là 615 g/cm² và khối lượng phân tử trung bình là 267 kDa. Phơ FT-IR đã cho thấy các dải hấp thụ mạnh ở 930 cm⁻¹ và 850 cm⁻¹ tương ứng với 3,6 anhydro-α-D-galactose liên kết ở vị trí 1,4 và β-D-galactose-4-sulfate liên kết ở vị trí 1,3 của kappa-carrageenan. Phơ ¹³C NMR đã chỉ ra các tinh hiệu cho các carbon anomer của β-D-galactose-4-sulfate ở 102,6 ppm và 3,6-anhydro-α-D-galactose ở 95,3 ppm. Phơ ¹H NMR đã cho thấy các đỉnh tần hiệu ở 3,57 ppm và 5,1 ppm tương ứng với proton O-methyl của 6-O-methyl-D-galactose liên kết ở vị trí 1,3 và proton α-anomer của 3,6 anhydro-α-D-galactose. Các kết quả này cho thấy rằng carrageenan từ rong đờ K. striatum là kappa-carrageenan với các nhóm disaccharide lập lại bao gồm 6-O-methyl-β-D-galactose-4-sulfate liên kết 1,3 và 3,6 anhydro-α-D-galactose liên kết 1,4 và không chứa iota-carrageenan. Vì vậy, rong này hứa hẹn sẽ là một nguồn giá trị để sản xuất carrageenan cho sự dụng trong thực phẩm hoặc y học.

Từ khóa: Carrageenophytes, cág trục, kappa-carrageenan, Kappaphycus striatum, tính chất