**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:** Carrageenomophytes, kappa-carrageenan, Kappaphycus striatum, properties, structure

**INTRODUCTION**

Carrageenan is extracted from red seaweed of the Rhodophyceae family commonly from genera such as *Eucheuma, Solieria, Cripus, Agardhiella, Chondrus, Hypnea, Sarconema* and *Iridaeae* (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-galactopyranose-4-sulfate and 4-linked 3,6-anhydro-α-D-galactose-4-sulfate.

**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.**
galactose-2-sulfate having two sulfate groups per disaccharide repeating unit. Lamda-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. G: 1,3-linked, β-D-galactose and DA: 1,4-linked 3,6-anhydo-α-D-galactose; R = R' = H, kappa-carrageenan; R = SO₃⁻, R' = H, iota-carrageenan; R' = CH₃, 6-O-methylated carrageenan (Craigie, 1990).

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. Rhodizone 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 powder.

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 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 rhodizonate 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, Craige (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 × 10⁻³ and α = 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)

323
spectrum of carrageenan sample was recorded on Bruker mode ALPHA at Nhatrang University.

$^{13}$C NMR and $^1$H NMR spectra of carrageenan sample were measured at 80°C with D$_2$O solvent on Bruker AVANCE 500MHz at Institute of Chemistry (VAST), using aceton as internal standard.

RESULTS AND DISCUSSION

Carrageenan yield, 3,6 anhydrogalactose and sulfate contents, gelling and melting temperatures, gel strength and viscosity-average molecular weight of kappa-carrageenan were shown in Table 1. The data in this study are in range of those reported for the red algae containing carrageenan with 3,6 anhydrogalactose content from 15 to 40%, ester sulfate from 23.1 to 34.5% (Hayashi et al., 2007; Nanaki et al., 2010; Zia et al., 2017), gelling temperature from 32.7 to 34.5°C, melting temperature from 52 to 56°C (Mendoza et al., 2002), gel strength from 503 - 1004 g/cm$^2$ (Le Dinh Hung et al., 2009), carrageenan content from 32.5 to 54.3% (Mendoza et al., 2002; Zuldin et al., 2016) and molecular weight from 100 to 700 kDa (Necas, Bartosikova, 2013). The differences in chemical composition and physical properties between carrageenan samples can be attributed to the extraction methodology used in each study, and the time of algal harvest.

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

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

a AG: anhydrogalactose; b MW: viscosity-average molecular weight. Mean ± SEM (n = 3).

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 O=S=O symmetric vibration and C$_4$–O–S stretching vibration, respectively. They stood for total –SO$_4$ and C$_4$–O–S of β-D-galactose, respectively. The band at 930 cm$^{-1}$ proved the existence of C–O–C of 3,6-anhydro-
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. \(^{13}\)C NMR spectrum of carrageenan extracted from \(K.\) striatum.](image)

| Unit                   | Carbon atom | Kappa-carrageenan | Ref \(^a\) | Ref \(^b\) | Ref \(^c\) |
|------------------------|-------------|-------------------|------------|------------|------------|
| D-Galactose-4-sulfate  | C-1         | 102.6             | 102.6      | 102.7      | 102.9      |
|                        | C-2         | 69.7              | 69.6       | 69.9       | 70.1       |
|                        | C-3         | 78.7              | 78.9       | 79.1       | 79.5       |
|                        | C-4         | 74.1              | 74.1       | 74.3       | 74.5       |
|                        | C-5         | 74.7              | 74.7       | 75.0       | 75.2       |
|                        | C-6         | 61.4              | 61.2       | 61.5       | 61.7       |
| 3,6-anhydro-D-galactose| C-1         | 95.3              | 95.2       | 95.4       | 95.7       |
|                        | C-2         | 69.9              | 69.9       | 70.1       | 70.4       |
|                        | C-3         | 78.9              | 79.1       | 79.4       | 79.6       |
|                        | C-4         | 78.4              | 78.2       | 78.5       | 78.7       |
|                        | C-5         | 76.7              | 76.7       | 77.0       | 77.2       |
|                        | C-6         | 69.4              | 69.4       | 69.7       | 69.9       |

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*.

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

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).
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. gelatinæ (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 alga 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 ĐÁC TÍNH CỦA KAPPA-CARRAGEENAN TỪ RONG ĐÔ KAPPAPHYCUS STRIATUM

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TÓM TẮT

Rong đō Kappaphycus striatum là loài rong kinh tế đang được nuôi trồng rộng rãi ở Việt Nam làm nguồn 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 sấy gel và 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 phần từ trung bình là 267 kDa. Phô FT-IR đã cho thấy các đại hợp thủy phân 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. Phô ¹³C NMR đã chỉ ra các tính hiệu cho các carbon anomé của β-D-galactose-4-sulfate ở 102,6 ppm và 3,6-anhydro-α-D-galactose ở 95,3 ppm. Phô ¹H NMR đã cho thấy các dinh dinh 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 đō 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 hướng đến làm một nguồn giá trị để sản xuất carrageenan cho sử dụng trong thực phẩm hoặc y học.

Từ khóa: Carrageenophytes, cẩu trúc, kappa-carrageenan, Kappaphycus striatum, tính chất