

# Chemical and physical characteristics of carrageenan extracted from *Eucheuma spinosum* harvested from three different Indonesian coastal sea regions

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## SUMMARY

Carrageenan extracted from *Eucheuma spinosum* harvested from three different coastal sea regions, where this alga has been mainly cultivated, were determined for their chemical and physical characteristics. The carrageenan was extracted from the seaweed using hot alkali followed by precipitation, drying, and milling. The carrageenan properties were determined in terms of yield, ash, mineral, sulfate content, functional group, molecular weight, and viscosity profile. Physical characteristics of carrageenan were evaluated by a texture analyzer for gel strength and a rapid visco analyzer for viscosity. The yield of carrageenan from Sumenep (34.81 ± 5.83%) and Takalar (37.16 ± 3.26%) was found to be relatively higher than that of Nusa Penida (25.81 ± 1.93%). The calcium content was higher than magnesium, potassium and sodium content, and no cadmium, lead, mercury, and arsenic detected in all carrageenan. The ash content was around 29%; while, the sulfate content was in the range of 30–32%, and those were not different in all carrageenan. The presence of sulfate content was identified by FTIR at absorption band of 1373 cm<sup>-1</sup>. It was found that the molecular weight of carrageenan from Takalar were relatively higher and the gel strength of carrageenan from Takalar were significantly higher than that of carrageenan from Nusa Penida and Sumenep. Likewise, upon cooling from 80 to 20°C, the viscosity profile of carrageenan from Takalar characterized by higher viscosity compared to that of carrageenan from Sumenep and Nusa Penida. These results indicated that carrageenan from Nusa Penida, Sumenep, and Takalar were identified as iota-carrageenan with similar physico-chemical characteristics except for the gel strength, viscosity profile upon cooling from 80 to 20°C and the yield.

**Key words:** *Eucheuma spinosum*, extraction, gel strength, iota-carrageenan, viscosity profile.

## INTRODUCTION

Indonesia produced around 3.3 million tons of seaweeds in 2010 and the seaweeds production increased to 8.3 million tons in 2014 (DGA-MMAF 2015). *Eucheuma spinosum* J. Agardh is one of the main cultivated seaweed species in the Indonesian marine environment, and it is one of the

sources of carrageenan production. This seaweed is mainly cultivated in the north coast of Nusa Penida island (Bali province) located between the coordinates of 8°40'53.54"S and 115°25'56.58"E to 8°46'11.94"S and 115°37'45.55"E; in the south coast of Sumenep in Madura island (East Java province) located between the coordinates of 7°07'58.01"S and 113°48'41.50"E to 7°07'59.42"S and 113°53'51.64"E; and in the west coast of Takalar (South Sulawesi province) located between the coordinates of 5°26'04.92"S and 119°13'53.31"E to 5°36'08.92"S and 119°27'28.98"E.

Research on carrageenan characteristics from *E. spinosum* in Indonesia is very limited, although several reports on seaweed aquaculture are available (Radiarta *et al.* 2014). However, characteristics of carrageenan from other *Eucheuma* species have been reported such as carrageenan of *Eucheuma isiforme* (C. Agardh) J. Agardh var. *denudatum* D.P. Cheney from Yucatan, Mexico (Freile-Pelegrin *et al.* 2006; Freile-Pelegrin & Robledo 2008), and other *Eucheuma* species from Florida (Dawes *et al.* 1977). Environmental factors are known to affect yield and quality of phycocolloid (Chopin *et al.* 1990; Piriz & Cerezo 1991; Brown 1995). This research was aimed to know whether the characteristics of carrageenan extracted from *E. spinosum* differed if cultivated from three different coastal regions in Indonesia.

Carrageenan has relatively high economic value because of its function as a stabilizer and texture-forming agent for food industry, as an agent for controlling the release of excipients and pharmaceutical compounds (Kranz *et al.* 2009), and as anti-tumor and immunomodulator, anticoagulant, and antiviral (Campo *et al.* 2009). However, the use of carrageenan in Indonesia is limited for food ingredient.

Carrageenan is usually extracted from seaweed with a hot alkaline extraction method (Van de Velde *et al.* 2001). The rate of extraction depends on temperature, alkaline concentration, ionic strength, and the specific alkaline media (Viana *et al.* 2004). Alkaline treatment is an important step in the

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production of carrageenan, and is commonly used to improve the gel strength of commercial products. During alkaline treatment, the six-sulfated units of carrageenan were converted to the corresponding 3,6-anhydrogalactose in *E. isiforme* (Freile-Pelegriñ & Robledo 2006). Around 70% of Indonesia's territory is covered by sea water resulting in a great complexity of its ecosystem. We assume that carrageenan obtained from seaweed cultivated from different specific geographical areas will have different chemical and physical characteristics, particularly of those related to hydrocolloid properties. Therefore, this research was conducted to study the characteristics of carrageenan extracted from *E. spinosum* in terms of extraction yield, the content of ash, minerals, and sulfate, FTIR spectra, molecular weight, gel strength, and viscosity profile.

## MATERIALS AND METHODS

### Materials

The seaweed used in this study was harvested after 45 days of cultivation from the following three different locations: the north coast of Nusa Penida island (Bali province); the south coast of Sumenep in Madura island (East Java province); and the west coast of Takalar (South Sulawesi province). The north coast of Nusa Penida island is a coral area with temperature ranges from 28 to 30°C, pH 7.5–8.5, and salinity 28–34 (Radiarta *et al.* 2013). The south coast of Sumenep in Madura island is a rocky beach with the average temperature of 30°C, pH 7–7.6, and salinity 30–36. The west coast of Takalar is also a rocky beach with the average temperature of 30°C. The collection of seaweed from the three locations was done during rainy season in January to February 2011.

The harvested seaweed was first cleaned in running water to remove dirt and excess of salt and then dried at room temperature of 27–29°C for 24 h until moisture content of the seaweed was around 20%. A part of the dried seaweed was sent to the Research Center Institute for Oceanography, Indonesian Institute of Sciences (LIPI-Ancol, Jakarta) for further taxonomical identification, while other parts were used for carrageenan extraction and analysis.

### Extraction of carrageenan

Carrageenan was extracted from the seaweed using a hot alkaline extraction method according to Rotbart *et al.* (1988) with some modification. One kilograms of dried seaweed was soaked in 50 L of water for 16 h. Furthermore, 0.2 g Ca(OH)<sub>2</sub> per g of seaweed was added and the seaweed was macerated at 60°C for 1 h. Extraction of carrageenan was performed at 95°C for 3 h with adequate stirring. Diatomaceous earth was further added at a concentration of 2% (w/v) followed by continuous stirring for 30 min, while the temperature was lowered to 80°C. The seaweed extract was filtered through a handmade filter press and the obtained filtrate was kept overnight. The pH of the filtrate was lowered from 12 to 9 by adding 5% HCl, and then 96% ethanol (1:1, v/v) was added to precipitate carrageenan. Carrageenan was collected, dried, and ground to a size of 60 mesh. The yield of extraction was calculated based on the weight of carrageenan powder divided by the weight of dried seaweed.

### Ash and mineral

One gram of carrageenan was incinerated in a muffle furnace at 550°C for 16 h and ash content was determined gravimetrically according to the method of AOAC, 930.05 (AOAC 2000). Mineral in carrageenan, in particular Ca, K, Mg, Na, Cd, Hg, Pb, and As were determined using atomic absorption spectrophotometer 6300 equipped with a single hollow cathode lamp (Shimadzu, Kyoto, Japan) for each element and an air-acetylene burner.

### Sulfate

One gram of carrageenan was weighed in an erlenmeyer and 50 mL 0.2 N HCl was added and heated to boiling for 1 h. After 1 h in boiling, 25 mL H<sub>2</sub>O<sub>2</sub> was added and heated for 5 h. This solution was transferred into a beaker and further heated to boiling. Furthermore, 10 mL 10% BaCl<sub>2</sub> was added and for 2 h. Precipitate formed upon boiling was filtered through an ashless filter (No. 42, Whatman, Munich, Germany) filter and washed with hot distilled water to remove the residual chloride. Filter paper containing precipitate was then dried and burned at a temperature of 700°C in a furnace. Ash was cooled in a desiccator and weighed until a constant weight was obtained. Sulfate content was calculated based on the following equation (JECFA 2007):

$$\text{Sulfate content(\%)} = \frac{P \times 0.4116}{\text{weight of sample}} \times 100$$

where; 0.4116 is a relative atomic mass of SO<sub>4</sub> divided by the relative atomic mass of BaSO<sub>4</sub>, and P is the weight of BaSO<sub>4</sub> precipitate (g).

### FTIR spectra analysis

About 4.0 mg carrageenan was homogenized thoroughly with 200 mg KBr in a mortar. The homogenized mixture was pressed to form a pill and used in FTIR spectra analysis. The infrared spectra of carrageenan was recorded on IR Prestige-21 spectrometer (Shimadzu, Kyoto, Japan) at a spectral resolution of 8 cm<sup>-1</sup>. Each spectrum of 45 scans was acquired at a resolution of 1 cm<sup>-1</sup>. A commercial grade, predominantly iota carrageenan (Type II SIGMA Aldrich, France) was used as standard.

### Molecular weight

The molecular weight of carrageenan was determined according to the method of Shiroma *et al.* (2008). The analysis was conducted using an HPLC (LC-6A, Shimadzu), on a 7.8 × 300 mm TSK-gel G5000 PWXL column (Tosoh, Tokyo, Japan), with a refractive index detector RID-6A (Shimadzu) at ambient temperature. The column was eluted with deionized water, at a flow rate of 0.5 mL min<sup>-1</sup>. Five pullulan molecular weight standards (Showa Denko K.K. Shodex, Kanagawa, Japan) were used as follows: P-10 (MW = 0.96 × 10<sup>4</sup>), P-20 (2.11 × 10<sup>4</sup>), P-50 (4.71 × 10<sup>4</sup>), P-100 (11.3 × 10<sup>4</sup>), P-200 (21.0 × 10<sup>4</sup>), P-400 (36.6 × 10<sup>4</sup>), and P-800 (80.5 × 10<sup>4</sup>). Carrageenan solution was prepared by

dissolving 10 mg carrageenan sample in 10 mL deionized water at 40°C.

### Gel strength

The gel strength of carrageenan was determined using a Texture Analyzer (TA-XT plus, Stable Microsystems, Goldaming, UK). Three grams of carrageenan sample was dissolved in 197 g of distilled water. The solution was then heated with regular stirring until the temperature reached 80°C. Hot solution was poured into a plastic container (diameter 6 cm, height 6.5 cm) and stored in a refrigerator at 10°C for 16 h before analysis (FMC 1977).

### Viscosity profile

Viscosity profile of carrageenan solution was determined using a Rapid Visco Analyzer (RVA-4, Newport Scientific Pty Techmaster, Ltd, Warriewood, Australia), upon cooling down the solution. A solution of 1% (w/w) carrageenan was prepared and heated in the RVA to temperature of 80°C with constant stirring at 160 rpm. The suspension was cooled down from 80 to 20°C at the rate of 1°C per min after a holding time at 80°C for 5 min and the changes in viscosity was monitored (Young 2003). The viscosity profile was indicated by a curve of viscosity increase against temperature is decreased.

### Statistical analysis

Data were analyzed using SPSS version 16 (SPSS, Chicago, IL, USA), and were treated statistically by one-way analysis of variance (ANOVA). Carrageenan characteristics from *E. spinosum* collected from Nusa Penida, Sumenep, and Takalar were compared using one-way ANOVA.

## RESULTS AND DISCUSSION

### Extraction of carrageenan

Hot alkali extraction of seaweed followed by alcohol precipitation and drying produced dried carrageenan with a yield ranged from 25.8 to 37%. The yield of carrageenan extraction of *E. spinosum* from Nusa Penida, Sumenep, and Takalar was  $25.8 \pm 1.93$ ,  $34.81 \pm 5.83$  and  $37.16 \pm 3.26\%$ , respectively (Table 1). The yield of carrageenan of *E. spinosum* from Sumenep and Takalar was relatively higher than that of Nusa Penida. The yield of carrageenan decreased slightly when the seaweed was treated with hot alkali process because of degradation of some polysaccharides (Stanley 1987). During a hot alkaline process sulfate groups will be bound in the cluster of galactose by sodium and potassium ions, and generate salts i.e.  $\text{Na}_2\text{SO}_4$  or  $\text{K}_2\text{SO}_4$  in solutions. Furthermore, dehydration will form anhydrous galactose polymer (Michael & Rideout 2001; Uy *et al.* 2005). Previous researcher (Freile-Pelegrin & Robledo 2006) reported the yield of carrageenan extracted from other *Eucheuma* species (*E. isiforme*) with the hot alkaline extraction method ranged from  $33.2 \pm 1.6$  to  $45.5 \pm 0.5\%$ .

The difference in carrageenan yield may be due to the differences in growing areas. Various factors in growing area may

influence the formation of carrageenan physiologically in the seaweed. The difference in environmental conditions such as water temperature, pH and salinity in coastal areas of Nusa Penida, Sumenep, and Takalar may affect the formation of carrageenan in the seaweed. Breden and Bird (1994) found that percent of gel yield and gel properties of carrageenan extracted from *Gymnogongrus griffithsiae* (Turner) C. Martius varied seasonally, and gel strength was correlated with water temperature. Previously, similar finding was reported by Wallner *et al.* (1992) that the carrageenan yield of *Hypnea musciformis* J. V. Lamouroux was significantly different if cultivated in different season in Brazil due to different environmental conditions. As an example, *H. musciformis* showed lowest carrageenan yield ( $22.7\% \pm 1.02$ ) in March 1988 and the highest values in September 1987 ( $34.88\% \pm 0.26$ ). Along with the water temperature, salinity was the other factor that influenced the daily growth rate and carrageenan yield and quality. It is one of the main environmental factors known to influence carrageenan yield and quality since the carrageenan is responsible for the ionic equilibrium of the cell (Percival 1979). The yield carrageenan was usually directly related to the growing environmental conditions, one of which is the intensity of light which plays an important role in the formation of carbohydrates (Zertuche-Gonzalez *et al.* 1993). Other factors that may influence the yield and quality of carrageenan are seaweed species.

### Chemical and physical characteristic

#### Ash and mineral content

The results show that ash content of carrageenan extracted from three different Indonesian coastal areas ranged from 28.26 to 29.03%. Mineral contents, in particular were calcium, sodium, magnesium and potassium (Table 1). Mineral content varies from one seaweed to the others. For example, a sample from Takalar contains 0.80% calcium, which is higher than that from Nusa Penida (0.46%) or Sumenep (0.68%). Similarly, a sample from Takalar contains 0.58% magnesium, which is higher than that from Sumenep (0.41%) or Nusa Penida (0.40%). In the case of potassium content, a sample from Nusa Penida contains more potassium (3.54%) than that from Sumenep (2.47%) or Takalar (2.88%). It is difficult to compare the values obtained for algal mineral composition with data in the literature because of environmental factors. Regarding the mineral content of carrageenan, it is clear that hot carrageenan extraction with  $\text{Ca}(\text{OH})_2$  will increase the calcium content of carrageenan as indicated. Typical toxic components such as cadmium, lead, mercury and arsenic were also analyzed; however, those levels were below the detection limit of the instrument.

#### Sulfate content

The results showed that carrageenan extracted from *E. spinosum* seaweed of Nusa Penida, Sumenep and Takalar contains 32.27, 30.74 and 31.94% of sulfate, respectively (Table 1). Sulfate content from Nusa Penida, Sumenep and Takalar is higher than the red seaweed *E. isiforme* from Yucatan and Nicaragua (19–26%) as reported by Freile-Pelegrin and Robledo (2008) and Freile-Pelegrin *et al.* (2006). Sulfate content determines the type of carrageenan fractions. Doty

**Table 1.** Characteristics of carrageenan collected *Eucheuma spinosum* from Nusa Penida, Sumenep and Takalar

	Cultivation area		
	Nusa Penida	Sumenep	Takalar
Yields of extraction (%)	25.81 ± 1.93	34.81 ± 5.83	37.16 ± 3.26
Ash (%)	29.03 ± 1.89	29.57 ± 0.04	28.26 ± 0.29
Minerals (%)			
Ca	4.84 ± 0.26	5.17 ± 0.04	5.14 ± 0.35
K	0.57 ± 0.11	0.67 ± 0.01	0.56 ± 0.06
Mg	0.66 ± 0.14	0.66 ± 0.04	0.33 ± 0.03
Na	1.84 ± 0.29	1.77 ± 0.09	1.46 ± 0.14
Sulfate (%)	32.27 ± 0.75	30.74 ± 0.56	31.93 ± 1.13
Molecular weight (10 <sup>5</sup> Da)	8.51 ± 1.01	8.40 ± 1.53	9.08 ± 1.54
Gel strength (g cm <sup>-2</sup> )	32.73 ± 1.61 <sup>a</sup>	43.30 ± 2.85 <sup>a</sup>	54.14 ± 1.79 <sup>b</sup>

Letters indicate statistical significance ( $P < 0.05$ ) for each location.

(1986) distinguished carrageenan into two fractions, namely kappa-carrageenan, which contains sulfate less than 28% and iota-carrageenan, which contains sulfate more than 30%. Based on the above criteria, all carrageenan from three different Indonesian coastal areas are found to be iota-carrageenan.

### Molecular weight of carrageenan

The molecular weight of carrageenan extracted from seaweed from Nusa Penida, Sumenep, and Takalar were  $8.51 \times 10^5$ ,  $8.40 \times 10^5$ , and  $9.01 \times 10^5$  Da, respectively. As a comparison, the molecular weight of iota-carrageenan standard was  $8.57 \times 10^5$  Da. The results show that molecular weight of all carrageenan extracted from three different regions are comparable with that of iota-carrageenan in general as reported by Tobacman (2001), which is in the range of  $3.00\text{--}8.00 \times 10^5$  Da. However, carrageenan from Takalar was found to be relatively higher in molecular weight compared to that from Nusa Penida or Sumenep. This finding seems to be in line with the results on the gel strength and viscosity profile.

### Gel strength

The gel strength of carrageenan from Nusa Penida, Sumenep, and Takalar were  $32.73 \pm 1.61$ ,  $43.30 \pm 2.85$  and  $54.14 \pm 1.79$  g cm<sup>-2</sup>, respectively (Table 1). Significantly, the gel strength of carrageenan from Takalar is higher than the other two. This significant difference in gel strength may be related to the fact that the molecular weight of carrageenan from Takalar was relatively higher than that from Nusa Penida or Sumenep. Previously, Freile-Pelegrin and Robledo (2008) reported that the gel strength of 1.5% carrageenan solution extracted from *E. isiforme* harvested from Nicaragua coast was less than 50 g cm<sup>-2</sup>. Our finding in the gel strength seems to be in line with their finding that the gel strength of iota-carrageenan is relatively weak.

### FTIR spectra

FTIR spectra of *E. spinosum* carrageenan extracted from the three region as well as the iota-carrageenan standard are

shown in Figure 1. Selected wave number (cm<sup>-1</sup>) of those spectra are tabulated in Table 2. Absorption band around 1373 cm<sup>-1</sup> indicates the presence of sulfate ester substitution in all carrageenan. While, absorption band at around 933 cm<sup>-1</sup> in all carrageenan are attributable to 3,6-anhydrogalactose residues. Absorption bands around 852 and 806 cm<sup>-1</sup> indicate the presence of axial sulfate ester substitution at the C4 and C2 of 3,6-anhydrogalactose, respectively. All absorption bands of the FTIR spectra (Table 2) show that carrageenan from the three Indonesian coastal areas are found to be iota-carrageenan. Such finding is in agreement with the work showing kappa and iota-carrageenan, which can be distinguished from all other carbohydrates because of the absorption band at 848 cm<sup>-1</sup> region as the D-galactose-4-sulfate, while the strong band at 806 cm<sup>-1</sup> is D-galactose-2-sulfate (Deslandes *et al.* 1990; Chopin & Whalen 1993).

### Viscosity profile of carrageenan

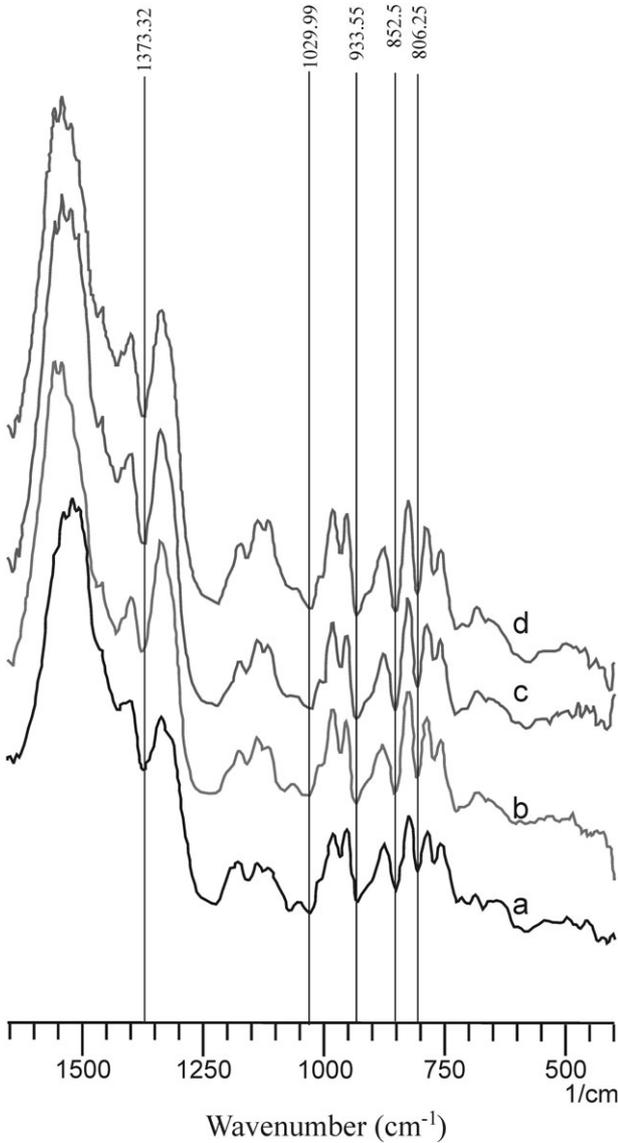
All carrageenan were soluble at 80°C with the same viscosity of around 120 mPa.s (Fig. 2).

When the carrageenan solution temperature was decreased to 20°C at a rate of 1°C min<sup>-1</sup>, there was no significant viscosity change detected for around 15 min until the temperature reached 65°C. Further cooling resulted in viscosity increase with different viscosity profile in the three carrageenans.

The viscosity of carrageenan from Takalar increased steadily and reached 600 mPa.s at a temperature of 42.5°C. While, carrageenan from Sumenep required a temperature of lower than 42.5°C, and carrageenan from Nusa Penida required even far below 42.5°C to reach the same viscosity of 600 mPa.s. At 20°C, the viscosity of carrageenan from Nusa Penida, Sumenep, and Takalar were 650, 1080, and 1200 mPa.s, respectively. The difference in viscosity profile of three carrageenans is in line with the yields of carrageenan extraction and the gel strength.

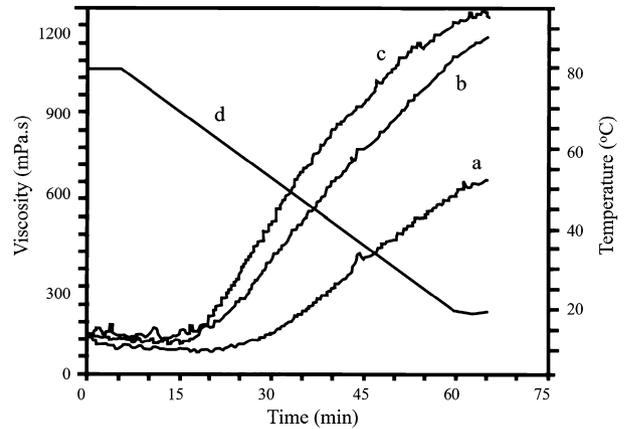
### CONCLUSION

Carrageenan extracted from *Eucheuma spinosum* harvested from Nusa Penida, Sumenep, and Takalar was identified as



**Fig. 1.** FTIR spectra of carrageenan from (a) Iota sigma, (b) Sumenep, (c) Nusa Penida, and (d) Takalar.

Iota-carrageenan with molecular weight ranged from  $8.40 \times 10^5$  to  $9.02 \times 10^5$  Da. The molecular weight of carrageenan from Takalar was relatively higher than that from Nusa Penida or Sumenep. The gel strength of the three



**Fig. 2.** Viscosity profile of carrageenan (80–20°C) from *Eucheuma spinosum* harvested at Nusa Penida, Sumenep and Takalar. (a) The viscosity profile of carrageenan from Nusa Penida (b) The viscosity profile of carrageenan from Sumenep (c) The viscosity profile of carrageenan from Takalar. (d) Temperature.

carrageenans ranged from 32.73 to 54.14 g cm<sup>-2</sup>. The gel strength of carrageenan from Nusa Penida and Sumenep was relatively the same; however, the gel strength of carrageenan from Takalar was significantly higher than that from Nusa Penida or Sumenep. Similarly, carrageenan from Takalar showed higher viscosity profile than carrageenan from Nusa Penida or Sumenep. We assume that the differences in gel strength and viscosity profile of carrageenan from three different Indonesian coastal sea regions may be influenced by their location characteristics and climate. It is difficult to have correct answer why the location characteristics and climate of the three coastal regions influence the gel strength and viscosity profile, because no data on carrageenan gel strength and viscosity profile from these locations are available. However, it was reported that the suitability of seaweed aquaculture, particularly *Kappaphycus alvarezii* Doty ex P.C. Silva in Nusa Penida (Radiarta *et al.* 2014), and Takalar (Akmal 2012) as well as *Eucheuma cottonii* Weber-van Bosse in Sumenep (Jailani *et al.* 2015), depends among others on water quality, which include dissolved oxygen, pH, salinity, nitrate, as well as air temperature, wind velocity, and sun light intensity. It is suggested that in-depth study may be needed to clarify the influence of *E. spinosum* growing conditions on the gel strength and viscosity profile of its extracted carrageenan.

**Table 2.** FTIR absorption bands of carrageenan from *E. spinosum* and Iota-sigma

Nusa Penida Wavenumber (cm <sup>-1</sup> )	Takalar Wavenumber (cm <sup>-1</sup> )	Sumenep Wavenumber (cm <sup>-1</sup> )	Iota-sigma Wavenumber (cm <sup>-1</sup> )	Functional groups
2912.51	2912.51	2912.51	2912.51	O—H
1373.32	1373.32	1377.17	1373.32	S=O (sulfate ester)
1080.14	1083.99	1083.99	1068.56	C—O (3,6-anhydrogalactose)
1029.99	1026.13	1026.13	1029.99	C—O—C (Glycosidic bound)
964.41	968.27	964.41	968.27	Galactose
933.55	933.55	933.55	933.55	C—O C—O (3,6 anhydrogalactose)
852.54	852.54	852.54	852.54	C—O—SO <sub>3</sub> at C4 3,6-anhydrogalactose 4-sulfat
806.25	806.25	806.25	806.25	C—O—SO <sub>3</sub> at C2 3,6-anhydrogalactose-2-sulfate

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