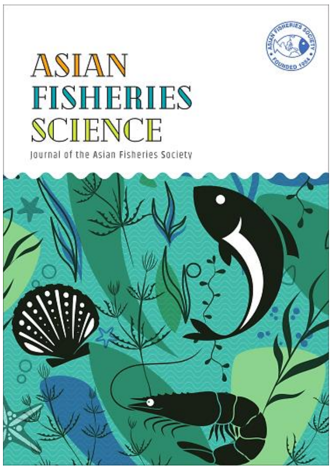

Characteristics of Semi-Refined Carrageenan From *Kappaphycus* Seaweed Farmed in Coastal Waters of Northern Java, Indonesia

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Abstract

Kappaphycus seaweed is the primary source of carrageenan, a polysaccharide widely utilised as a thickening and gelling agent in many commercial products. Unlike pure refined carrageenans, semi-refined carrageenans (SRC) are considered more cost-efficient and easier to produce. This study evaluates the quality of SRC extracted from *Kappaphycus alvarezii* (Doty) Doty ex Silva 1996, and *Kappaphycus striatus* (F. Schmitz) Doty ex P.C. Silva 1996, seaweeds farmed in the northern part of Java Island, particularly in Serang, Seribu Islands, and Karimunjawa Islands. Using 8 % KOH as a solvent, SRC was extracted from clean dried seaweed. Physical and chemical characteristics of the SRC were measured by assessing the percentage SRC yield, ash content, moisture, sulphate content, viscosity (cp) and gel strength (g.cm^{-2}). Pearson's correlation analysis was performed to investigate the relationship between SRC quality and environmental factors. Fourier transform infrared spectroscopy (FTIR) analysis showed that all samples had kappa-carrageenan from peak absorbance of 1218–1224 cm^{-1} indicating ester sulphate bonds, 924–925 cm^{-1} indicating 3,6-anhydrogalactose, and 843–844 cm^{-1} suggesting galactose-4-sulphate groups. Furthermore, ash content, moisture, and viscosity of SRC passed the FAO standard, while the sulphate content was below the threshold limit. Several environmental factors had a significant correlation with SRC quality, such as water pH positively correlated with gel strength, salinity level positively correlated with gel strength, and depth positively correlated with ash content. This study concluded that SRC produced from the sampling sites had varying qualities and may be affected by environmental parameters in their cultivation site.

Keywords: FTIR, gel strength, viscosity, SRC quality

Introduction

Carrageenan is a derivate product extracted from the cell walls of red seaweed that forms a linear polysaccharide with repeated units of galactose and 3,6-anhydrogalactose (Rao et al., 2019) linked by alpha-1-3 and beta-1-4 glycosidic linkages (Kulkarni and Shaw, 2016). Carrageenans are extensively employed as a gelling and thickening agent in various products (Naseri et al., 2019). There are various varieties of carrageenans based on their gelling and thickening properties, with kappa, iota, and lambda carrageenans being the most commercially popular. Both kappa and iota carrageenan can form a gel in the presence of a specific ion (i.e., potassium ion for kappa and calcium ions for iota), while lambda carrageenan solely acts as a thickening agent (Pereira, 2018).

Rhodophytes or red seaweeds are considered the primary source of carrageenan, particularly from the genus *Chondrus*, *Gigartina*, *Mastocarpus*, *Sarcothalia*, *Betaphycus*, *Kappaphycus*, and *Eucheuma* (Pereira et al., 2009). Some of them, such as *Kappaphycus* and *Eucheuma*, have been massively cultivated worldwide for their carrageenan properties. *Kappaphycus* has been the more commonly cultivated seaweed because of its kappa-type carrageenan, which has a larger market than the other types of carrageenan (Bixler and Porse, 2011). Indonesia, Malaysia, and the Philippines have been the leading producers of cultivated *Kappaphycus* seaweed (Hurtado et al., 2014). The cultivated species are mainly *Kappaphycus alvarezii*

(Doty) Doty ex Silva, 1996, also known as cottonii seaweed and *Kappaphycus striatus* (F. Schmitz) Doty ex P.C. Silva, 1996, which is called sacol seaweed. In Indonesia, *Kappaphycus* seaweed is mainly cultivated in the Sulawesi region, Bali, Nusa Tenggara, and Java Island.

Farmers typically sun-dry the cultivated *Kappaphycus* seaweed before selling it to carrageenan manufacturers. The final product is usually refined carrageenan (RC), a pure form of carrageenan extracted from seaweed and free of impurities. Aside from RC, there is another alternative product called SRC or semi-refined carrageenan. While RC is produced by extracting carrageenan from seaweed by cooking and precipitating it in ethanol, SRC is made by cooking the seaweed in a hot alkali solution at a particular temperature, followed by neutralising, drying, and milling process (Chairani et al., 2019). As a result, SRC is cheaper to produce yet still has a similar overall application to refined carrageenan. The main difference is that SRC still contains impurities such as cellulose since the product itself was made without actually extracting carrageenan from the cell wall (McHugh, 2003).

SRC has been categorised as carrageenan by FDA regulation and EU regulation under E407a, while refined carrageenan is categorised as E407 (EFSA ANS Panel et al., 2018). The main difference is the presence of cellulose in the SRC powder since the product itself is made without extracting carrageenan from the seaweed cell wall. The residual cellulose content in SRC makes the end product cloudy upon its application, and hence it is often used in products where clarity is not an issue. SRC is now mainly used in the meat industry to extend the meat and other protein sources, increasing the value of the end product (Hotchkiss et al., 2016).

Different coastal waters could affect the growth and quality of cultivated seaweed (Parakkasi et al., 2020), which in turn could affect their carrageenan product, particularly SRC. This study aims to evaluate the physical and chemical characteristics of SRC extracted from *K. alvarezii* and *K. striatus* seaweed species farmed on the northern coast of Java Island, particularly in Serang, Seribu Islands, and Karimunjawa Islands. Dried seaweed samples were collected from six locations (Serang, Seribu Islands, and three locations in Karimunjawa). The carrageenan content, gel strength, viscosity, moisture, ash, and sulphate content of SRC obtained from each location was assessed, molecular characteristics were analysed using Fourier transform infrared spectroscopy (FTIR) method.

Materials and Methods

Sample collection

Seaweed sampling sites are depicted in Figure 1.

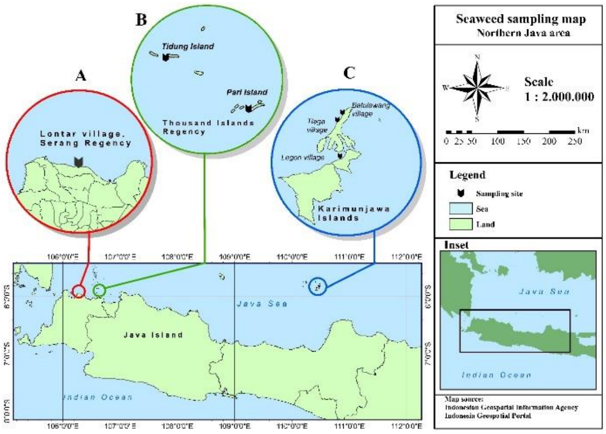

Fig. 1. Seaweed sampling sites. A: Serang Regency region, B: Thousand Islands Regency region, C: Karimunjawa Islands region.

Dried seaweed samples were collected from Lontar village in Serang (5°57'52.3"S, 106°17'52.2"E), Pari Island (5°51'32.8"S, 106°36'37.2"E) and Tidung Island (5°48'07.6"S, 106°31'05.6"E) in Seribu Islands Regency, and three villages in Karimunjawa Islands, namely Tlaga village (5°46'58.3914"S, 110°28'10.1723"E), Batulawang village (5°46'32.6428"S, 110°28'27.7788"E), and Legon village (5°49'4.1311"S, 110°28'19.6918"E).

The seaweeds had been previously identified as *K. alvarezii* from all locations except for *K. striatus* samples from Tidung Island, characterised by bushy cauliflower-like branching. All seaweeds were bought from local farmers after 40 days of cultivation. Environmental parameters such as water temperature, pH, and salinity were measured in situ. The temperature was measured using a liquid-in-glass thermometer, pH was measured using a pH meter (pHep HI 98107, Hanna, USA), salinity was measured using a hand refractometer (Atago, Japan), and depth was measured using a weighted measuring tape.

Preparation of seaweed

Samples of dried seaweed from each location were cleaned before being processed. The dried seaweed was separated according to sampling locations, soaked in tap water for 15 min and washed in running water to remove impurities. Hydrated seaweed was then cut into 2–4 cm pieces and sun-dried for 3 days.

SRC processing

The SRC production process was based on the KOH solution method (Rizal et al., 2016) with modifications. Dried clean seaweed sample from each location was weighed to 30 g and cooked in 300 mL (1:10 ratio) of 8 % (w/v) KOH solution in 80 °C (± 3 °C) for 120 min using a water bath. The cooked alkali-treated seaweed was then strained, washed in running water to remove excess KOH from the surface, and soaked in 2 L of distilled water overnight to neutralise the pH to 8–9.

The alkali-treated seaweed was chopped to 1–2 cm bits using a chopper, air-dried for 3 days, and further dried in the oven at 60 °C for 24 h before the milling process. Dried alkali-treated seaweed bits were milled using a grinder to produce SRC powder and sieved through 1 mm mesh. SRC yield was calculated using the formula:

$$\text{Yield SRC (\%)} = \frac{\text{Weight of SRC (g)}}{\text{Weight of dry seaweed (g)}} \times 100\ \%$$

Gel strength

Gel strength was measured by applying mechanical stress to SRC gel with KCl solution and distilled water. SRC gel for water gel strength analysis was prepared by dissolving 1.5 g of SRC powder in 100 mL of distilled water and heated in a water bath at 90 °C for 20 min with constant stirring. For KCl gel strength analysis, SRC powder was dissolved in 100 mL of 1.5 % KCl solution before heating, followed by the same steps. The gel solution was then left to cool, sealed with aluminium foil, and stored at 4 °C overnight. Finally, gel strength was measured by testing the mechanical resistance of the SRC gel using a texture analyser (Brookfield CT3 Texture Analyzer, USA).

Viscosity

About 1.5 g of SRC powder was dissolved in 100 mL hot water at 90 °C and stirred for 20 min. The SRC solution was then measured for its viscosity level using Brookfield DV1 digital viscometer (Bono et al., 2014).

Chemical quality analysis

Moisture content was measured with a moisture analyser (OHAUS MB120, China) using 500 mg of fine SRC sample. Ash content was measured using the gravimetric method (AOAC, 2006). Sulphate content was measured using the sulphate hydrolysis method (Dewi et al., 2012). Sulphate analysis was conducted by dissolving 1 g of SRC powder in 50 mL of 0.2 mol.L^{-1} HCl solution and boiled for 6 h. After the boiling process, 10 mL of $BaCl_2$ was added to the solution and boiled again for 2 h. The solution was then left to cool at room temperature for 4 h. The formed precipitate was filtered using GFF Whatman filter paper and incinerated in a furnace at 1000 °C for 1 h. The resulting white ash was then weighed. Sulphate content was calculated using the following formula:

$$\%\ \text{Sulphate} = \text{BaSO}_4\ \text{precipitate (g)} \times 100\ \% \times 0.4116$$

FTIR analysis

Surface response analysis was conducted using FTIR spectrometer (MB300, ABB, Canada) in the Central University Laboratory of Universitas Gadjah Mada. SRC samples were finely ground and sieved to ensure fine particles. About 2 mg of SRC samples were mixed with 200 mg of KBr and pressed to form homogenous pellets and immediately put to the sample plate of the spectrometer. Spectra bands were recorded in the range of 4000–650 cm^{-1}.

Data analysis

Characteristic parameters of SRC were statistically analysed using One-way ANOVA with 95 % confidence interval followed by Tukey HSD post hoc test with Minitab 19 software to determine the statistical significance of different SRC quality parameters at each location. Pearson correlation analysis was used to determine the correlation coefficients between environmental factors (temperature, salinity, pH, and depth) and physical-chemical quality of SRC (% yield, KCl gel strength, water gel strength, viscosity, ash content, and sulphate content). Linear regression analysis was performed between environmental parameters and SRC quality parameters with a statistically significant correlation. FTIR result was analysed descriptively.

Results

Environmental parameters in cultivation site

Water quality parameters measured in this study included temperature, salinity, and pH (Table 1). The depth and substrate of the cultivation site were also assessed. The water temperature at the cultivation sites ranged from 27 to 29 °C, salinity from 28 to 35 ‰, and pH from 7.9 to 8.5. The water parameter was considered adequate in accordance with Indonesian national standard guideline (SNI) for *Eucheuma cottonii* (*K. alvarezii*) cultivation, where the acceptable range for water temperature is between 26–32 °C, salinity level between 28–34 ‰, and pH level around 7–8.5 (SNI, 2010).

The cultivation sites in all sampling locations were in shallow coastal waters with less than 5 m depth. Most *K. alvarezii* farms used the long-line cultivation method, while *K. striatus* in Tidung Island was farmed with the off-bottom method in the lower intertidal area of the beach. The substrate in the farm site was mostly sand, except in Lontar village, Serang, and Legon village, Karimunjawa, where the substrate is sandy mud. Both cultivation sites were located near the mangrove area, hence the muddy substrate.

Physical and chemical characteristics of SRC

Figure 2 shows the SRC yields from each sample. The average SRC yield percentage obtained from the six samples ranged between 25.02 ± 1.13 % to 39.79 ± 1.12 %. ANOVA analysis showed statistically significant difference in SRC yield between locations (F(5,12) = 7,

Table 1. The environmental parameters at the seaweed cultivation sites.

Location	Species	Temperature (°C)	Salinity (‰)	pH	Depth (m)	Substrate
Serang	*Kappaphycus alvaezii*	27.5–29.0	28.0–29.0	7.8–7.9	1.3	Sandy mud
Pari Island	*Kappaphycus alvaezii*	27.0–28.0	30.0–32.0	8.0–8.2	3.0	Sand
Tidung Island	*Kappaphycus striatus*	28.0–29.0	32.0–33.0	8.2–8.3	0.6	Sand
Tlaga village	*Kappaphycus alvaezii*	27.0–29.0	32.0–34.0	8.3–8.5	3.0	Sand
Batulawang village	*Kappaphycus alvaezii*	27.0–28.0	33.0–35.0	8.2–8.4	3.0	Sand
Legon village	*Kappaphycus alvaezii*	27.0–28.0	33.5–34.0	8.0–8.1	4.0	Sandy mud

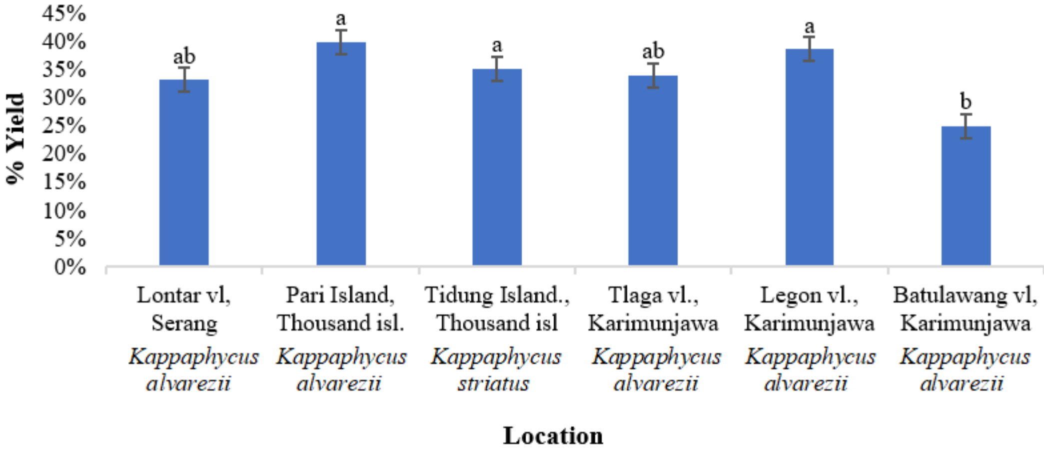

Fig. 2. The percentage yield of semi-refined carrageenans (SRC) produced by seaweed samples from each sampling location. Values with different lowercase letters indicate statistical significance ($P < 0.05$).

$P = 0.003$, $P < 0.05$). Post hoc Tukey test showed that SRC yield from Lontar and Tlaga villages (*K. alvarezii*) were statistically different from samples from Pari Island (*K. alvarezii*), Legon village (*K. alvarezii*), and Tidung Island (*K. striatus*). In contrast, the Batulawang sample (*K. alvarezii*) was significantly different compared to other samples. The sample with the highest SRC yield was *K. alvarezii* seaweed from Pari Island (39.79 ± 1.12 %), while the lowest yield was obtained from Batulawang village, Karimunjawa (25.02 ± 1.13 %). Legon village sample had the highest SRC yield compared to two other samples from Karimunjawa (38.54 ± 1.11 %).

The gel strength parameter of SRC in both water and KCl solutions are presented in Figure 3. Water gel strength ranged from 20.98 ± 0.2 g.cm^{-2} to 83.55 ± 5.2 g.cm^{-2} at all locations. There was a significant difference between locations (F (5,12) = 46.36, P = < 0.001). The highest gel strength was obtained from Tlaga sample (83.55 ± 5.2 g.cm^{-2}) while the lowest water gel strength was from Serang sample (20.98 ± 0.2 g.cm^{-2}). KCl gel strength had higher values compared to water gel strength, ranging from 380.25 ± 27.05 to 614.75 ± 26.05 g.cm^{-2} and there was statistical significance between locations (F (5,12) = 62.02, P = < 0.001).

Compared to the result of water gel strength, SRC from the Tlaga sample showed the highest value (614.75 ± 26.05 g.cm^{-2}). SRC from the Serang sample had the second-highest gel strength value (520.05 ± 0.95 g. cm^{-2}), significantly higher compared to its water gel strength value which is considered the lowest among the other samples. On the other hand, Batulawang SRC had the lowest KCl gel strength value (380.25 ± 27.05 g.cm^{-2}).

Viscosity levels of SRC solution for each sample are shown in Figure 4. Viscosity of SRC solution ranged from 28.5 ± 1.52 – 125 ± 3.05 cp with significantly different value from most location (F (5,12) = 189.88, P = < 0.001). The highest viscosity level was obtained from the Legon sample (125 ± 3.05 cp), while the lowest viscosity was from the Batulawang sample (28.5 ± 1.52 cp). Tlaga sample had a lower viscosity value than most samples (59 ± 4.51 cp), yet it had the highest gel strength. On the contrary, the Legon sample had the highest viscosity value but lower gel strength than most samples.

The chemical properties of SRC from each sample are shown in Figure 5. The chemical properties analysed in this study were sulphate content, ash content, and moisture content.

Moisture content from SRC samples ranged between 6.70 ± 0.09 to 8.01 ± 0.2 %, with significant difference between locations (F (5,12) = 6.01, P = 0.005, P < 0.05). SRC derived from Tlaga village (Karimunjawa) had the

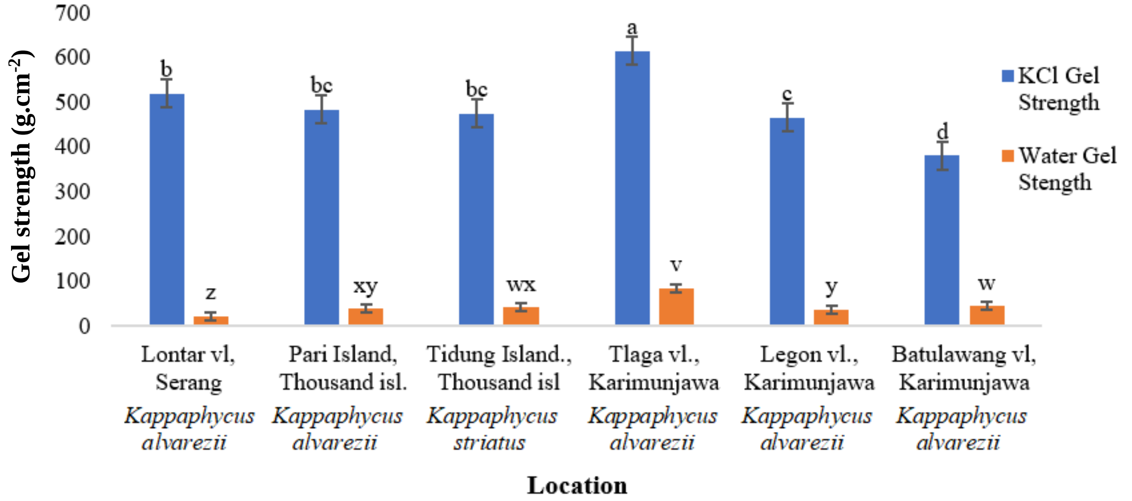

Fig. 3. Gel strength from KCl solution and water solution (g.cm^{-2}) of semi-refined carrageenans (SRC) samples from each location. Values with different lowercase letters indicate statistical significance ($P < 0.05$).

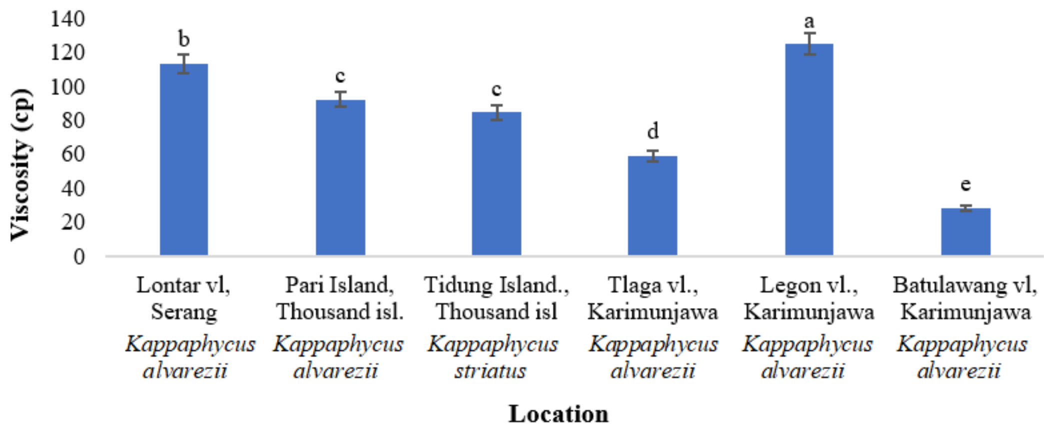

Fig. 4. Viscosity value (cp) from semi-refined carrageenans (SRC) from each location. Values with different lowercase letters indicate statistical significance ($P < 0.05$).

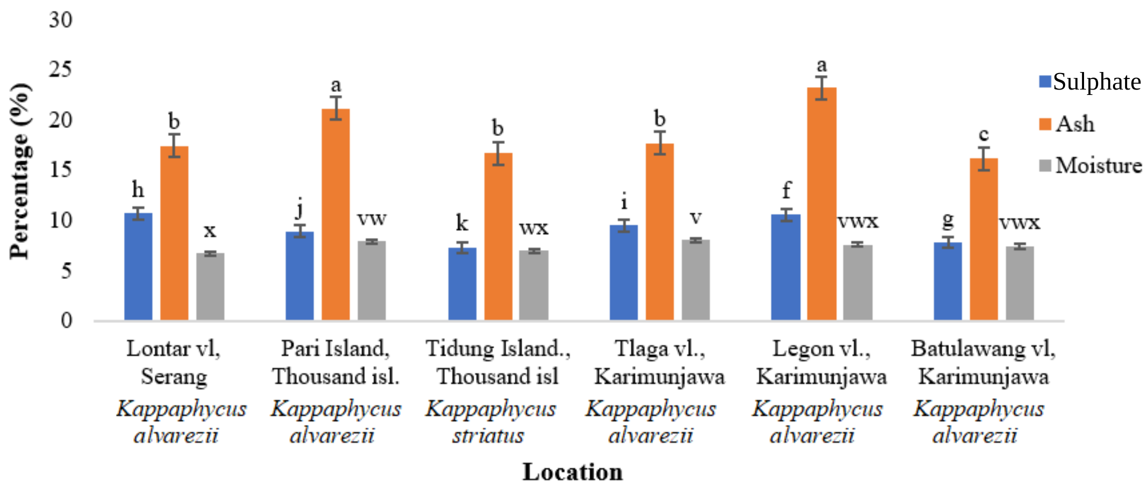

Fig. 5. Chemical properties of semi-refined carrageenans (SRC), measured as percentage of ash content, moisture content, and sulphate content. Values with different lowercase letters indicate statistical significance ($P < 0.05$).

highest moisture content (8.01 ± 0.2 %), followed by SRC from Pari Island (7.88 ± 1.07 %). SRC from Serang had the lowest moisture content (6.70 ± 0.09 %).

Ash content in SRC samples ranged from 16.7 ± 0.16 to 23.20 ± 0.11 % with statistically significant difference between locations (F (5,12) = 35.42, P = < 0.001). SRC from Legon village and Pari Island had the highest ash content (23.20 ± 0.11 % and 21.12 ± 0.06 %, respectively), both values are statistically different.

Sulphate is one of the main components of carrageenan structure. Sulphate content found in this study ranged from 7.28 ± 0.10 to 10.69 ± 0.07 % with statistically significant difference between locations ($F(5,12) = 632.68$, $P = < 0.001$). SRC sample from Serang had the highest sulphate content (10.69 ± 0.07 %), while the lowest was from the Tidung island sample (7.28 ± 0.10 %). Post hoc statistical tests showed that the sulphate content in each location was statistically different.

The overall difference between the physical quality of SRC between *K. striatus* from Tidung Island and *K. alvarezii* from other locations, despite statistical difference, did not particularly stand out. In terms of yield percentage, gel strength, and viscosity level, the quality values of *K. striatus* SRC were similar to *K. alvarezii* from Pari Island, both from Thousand Islands regency. SRC from *K. striatus* had the lowest sulphate value compared to other samples. However other chemical qualities did not show exceptional values despite statistical difference between samples.

Correlation between the physical and chemical quality of SRC and environmental factors from all sampling sites is presented in Table 2.

Table 2. Pearson correlation coefficient (r) between semi-refined carrageenans (SRC) quality (yield (%), viscosity (cp), KCl gel strength (g.cm^{-2}), water gel strength (g.cm^{-2}), ash content (%), sulphate content (%)) and environmental factor (pH, temperature, salinity, and depth).

Quality parameter	pH	Temperature	Salinity	Depth
SRC yield	-0.258	-0.033	-0.174	0.103
Viscosity	-0.671*	0.041	-0.394	-0.008
KCl gel strength	0.031	0.278	-0.269	-0.082
Water gel strength	0.698*	0.062	0.517*	0.286
Ash	-0.423	-0.350	0.102	0.648*
Sulphate	-0.711*	-0.083	-0.331	0.400

*Significant difference at $P < 0.05$.

Correlation analysis showed no linear correlation between environmental parameters with SRC yield and KCl gel strength ($P > 0.05$). Strong negative correlation was observed between pH and viscosity (r = -0.671), as well as sulphate content (r = -0.711). A strong positive correlation was shown between pH value and water gel strength (r = 0.698). Another strong positive correlation was indicated between depth and ash content (r = 0.648) and between water gel strength and salinity (r = 0.517).

Linear regression analysis identified pH contributed to 45 % of the observed variation of viscosity ($R^2 = 0.45$, $P = 0.002$), and 50.6 % of sulphate content ($R^2 = 0.506$, $P = 0.001$). The pH value also explained 48.7 % of the observed variance in water gel strength parameter ($R^2 = 0.487$, $P = 0.001$). Salinity levels contributed 26.7 % ($R^2 = 0.267$, $P = 0.028$) to observed variance of water gel strength. Water depth contributed to 42 % ($R^2 = 0.42$, $P = 0.004$) variation in the observed ash content.

FTIR spectra analysis

The result of FTIR spectra analysis is presented in Figure 6. The peaks in the absorption spectra indicate molecular bonds that can be used to identify the presence of a specific molecular structure. All samples had a similar pattern of peak absorption at fingerprint region 600–1500 cm^{-1}, i.e., peak between 1218–1224 cm^{-1}, 924–925 cm^{-1},1029–1034 cm^{-1}, and 843– 44 cm^{-1}.

Discussion

Physical and chemical quality of SRC

This study investigated the difference in physical and chemical quality of SRC made from cultivated *Kappaphycus* seaweed in several northern coastal areas of Java. The results showed that most *Kappaphycus* seaweeds that came from a different location with different environmental parameters produced distinct qualities of SRC. Samples were

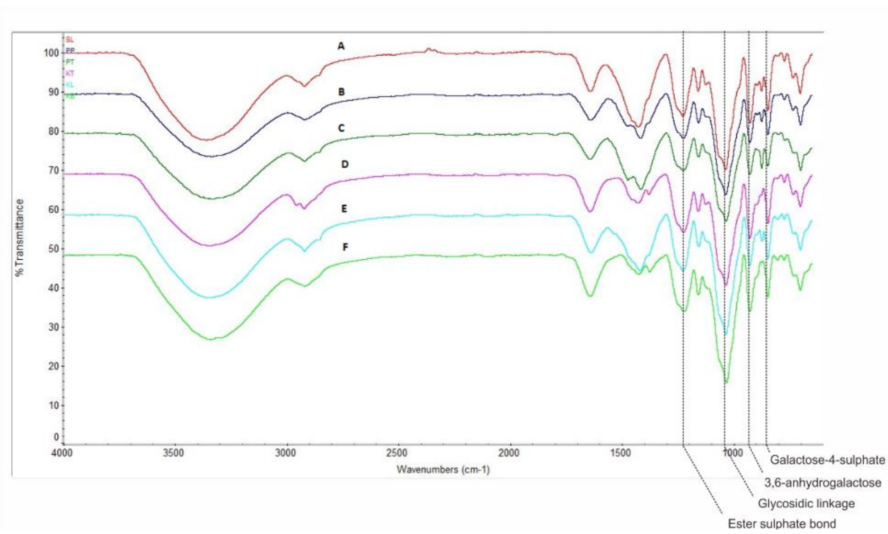

Fig. 6. Fourier transform infrared spectroscopy spectra from semi-refined carrageenans samples. (A) Serang (*Kappaphycus alvarezii*), (B) Pari Island (*Kappaphycus alvarezii*), (C) Tidung Island (*Kappaphycus striatus*), (D) Tlaga village (*Kappaphycus alvarezii*), (E) Legon village (*Kappaphycus alvarezii*), (F) Batulawang village (*Kappaphycus alvarezii*).

collected in October – November of 2019 when it was still in the dry season, and hence there was sufficient irradiance for seaweed cultivation, and drying was not affected by rainfall.

The SRC was produced using the alkaline treatment method with potassium hydroxide (KOH). KOH increased gel strength by converting the precursor carrageenan to kappa carrageenan while reducing the sulphate group in the carrageenan structure (Distantina et al., 2011). Hydroxide (OH^-) ions reduced the amount of ester sulphate group and increased 3,6-anhydrogalactose (3,6-AG), while the alkaline treatment also removes pigment, proteins, and other impurities (Anisuzzaman et al., 2013). Carrageenan gel strength was enhanced as the 3,6-AG content was increased.

Alkaline treatment was conducted by cooking the seaweed in elevated temperature (80 °C) for 120 min. Carrageenan structure was formed as a random coil at high temperatures due to electrostatic repulsion between polymer chains (Campo et al., 2009). Due to its thermo-reversible nature, the polymeric chain changed its formation to a more stable helix structure upon cooling (Thrimawithana et al., 2010). Furthermore, cations (K^+) in the alkaline solution would aggregate the helical dimers and form a stable three-dimensional network. As a result, a stronger polymer network was formed through intermolecular interactions between the carrageenan chains (Gulrez et al., 2011).

The main idea of SRC production is to enhance the gelling capacity of seaweed by cooking it in alkaline solvent but does not extract the carrageenan from the cell wall. Subsequently, the cellular materials were still retained and added up to the total SRC mass. Cellulosic materials and residual debris from the seaweed cell wall constituted 20–30 % of SRC (Ghosh et al., 2006).

The water quality parameters in the cultivation sites were mainly in the optimum range for *Kappaphycus* growth. Surface water temperature measured between 27–29 °C in daylight. The temperature range of 23–30 °C positively correlates with high biomass and the growth of *K. alvarezii* (Kumar et al., 2015). On the other hand, warmer water temperature (>32 °C) negatively affects the growth of *Kappaphycus*, decreased pigment content, and carrageenan yield of *K. alvarezii* (Kumar et al., 2020).

Salinity levels in the cultivation area ranged from 28–35 ‰. Serang waters had the lowest salinity level (28–29 ‰) because of their proximity to the mouth of a small river near the location. The salinity level in Batulawang waters (33–35 ‰) was slightly above the optimum threshold set by National Standard Guideline (SNI, 2010). However, previous studies reported that carrageenan yield from *K. alvarezii* grown in 35 ‰ salinity had the same quantity as the typical yield obtained from that species (Hayashi et al., 2011). Aside from the carrageenan, salinity affects the survivability and growth of *Kappaphycus* seaweed. The salt content in seawater plays a significant role in seaweed osmoregulation (Harwinda et al., 2018).

Other chemical factors such as water acidity or pH level in seawater also affect the growth of *Kappaphycus* seaweed. The pH level found in this study (7.8–8.5) is in the acceptable range for cultivation (7–8.5) (SNI, 2010). Lower pH levels could decrease seaweed growth rate (Tee et al., 2015). The suitability of environmental parameters is crucial in seaweed cultivation because seaweed's health in the water environment reflects its productivity and quality (Ateweberhan et al., 2015). The SRC yield obtained in this study was generally higher than the previous study, with 29.89–32.55 % yield using a similar alkaline method (Moses et al., 2015). The lowest SRC yield was obtained from *K. alvarezii* in Batulawang village in Karimunjawa, yet the water quality parameters are similar to two other samples in the same area. Other factors aside from water quality that could be accounted for are the harvest age and growth period (Periyasamy et al., 2019) and seedling quality (Aeni et al., 2019). The optimal harvest age for higher carrageenan content in *Kappaphycus* seaweeds was after 40 days of cultivation. Lower carrageenan content could indicate an inadequate cultivation period.

A previous study found that *K. alvarezii* had a higher gel strength value than other *Kappaphycus* seaweeds, although the value was insignificant (Adharini et al., 2019). The gel strength value of kappa-carrageenan in SRC is enhanced when cooked in the presence of specific monovalent or divalent cations. The cations bind with negatively charged ester sulphate groups, creating intermolecular interaction between the carrageenan molecular structure and forming gelation in a cooled state (Rhein-Knudsen et al., 2015).

Higher KCl gel strength compared to water gel strength was due to the presence of potassium cation (K^+) counterions. The addition of KCl in the carrageenan solution promotes gelling by cross-linking the polymer chain of carrageenan, forming cohesive networks of three-dimensional double helices structure with sulphate groups aligned outward (Thrimawithana et al., 2010). Aside from K^+, other cations such as Ca^+ are also capable of promoting gelation and increasing gel strength (Campo et al., 2009).

KCl gel strength value was comparable to another study that added 0.2 % KCl solution to SRC, which produced gel strength values between 526–650 $g.cm^{-2}$ (Moses et al., 2015). According to the previously mentioned research, the gel strength level is affected by the parameters used in the cooking process of SRC, such as the concentration of alkaline solvent (KOH).

The result of water gel strength measurement in this study was found to be lower than the gel strength measured in the previous research by Anisuzzaman et al. (2014), which also tested gel strength without adding counterions. The lower gel strength may be due to the additional step of soaking the cooked seaweed in distilled water overnight to reduce the pH level. Inorganic ions such as potassium cations that would act as counterions may be washed in the soaking process, therefore lowering the gelation ability of the SRC solution.

The overall viscosity of SRC was higher than the value set by FAO (more than 5 cp) (McHugh, 2003). The highest viscosity value is slightly higher than the viscosity result from a previous study that used a similar alkaline cooking process (higher value of 121.6 cp and the lowest of 60.80 cp) (Sormin et al., 2018). Viscosity levels were also higher than the values reported by Manuhara et al. (2016) with KCl addition. Viscosity level is affected by the method of SRC processing, such as cooking time, temperature, and solvent concentration (Bono et al., 2014).

Sulphate value was lower than FAO standard for carrageenan (15 %–40 % of dry weight). Lower sulphate content in SRC was also reported by previous research (Heri et al., 2018). According to Chan et al., (2013), commercial kappa-carrageenan contained 12.00–19.71 % of sulphate content. Moisture content from SRC samples ranged between 6.70 ± 0.09 to 8.01 ± 0.2 %, which complied with the range value of lower than 12 % as designated by FAO. Moisture content was influenced by the drying method of SRC. In this study, the alkali-treated seaweed chips produced by cutting the cooked seaweed were sun-dried for 3 days. Before the milling process using a grinder, the dried chips were further dried in the oven at 60 °C for 24 h. Dried chips made it more brittle and easier to grind. The extra oven drying step further lowers the moisture content of the resulted SRC.

The overall ash content is within the range of the standard value for carrageenan products by FAO (15–40 % of dry weight). Ash content represents the total mineral content of algae biomass (Liu, 2019). Washing and cleaning the dried seaweed should remove excess minerals, salts, and other impurities, lowering the ash content in processed seaweed. The alkaline treatment process may increase the ash content in carrageenan, but a longer cooking time might reduce it due to the higher number of cations that interact with the sulphate group of carrageenan (Astuti et al., 2017). Factors such as the addition of KOH solvent, cooking temperature, seaweed washing and cleaning process, and the purity of the water used to soak the alkalised seaweed could affect the ash content of SRC products (Basmal and Ikasari, 2014).

The present study found a correlation between several environmental parameters and SRC quality. For instance, water pH was negatively correlated with the viscosity and sulphate content of SRC. In contrast, water pH positively correlated with water gel strength. *Kappaphycus alvarezii* grown in a low acidity medium was found to have decreased levels of carbon (CO_2) accumulation (Tee et al., 2015). Thus, a low carbon source in an acidic medium might decrease the biosynthesis of cellular polysaccharides, which in turn would decrease the gel strength of carrageenan.

Water gel strength was also positively correlated with salinity. Optimum salinity is crucial for maintaining cellular integration and metabolic activities (Araújo et al., 2014). Optimum thallus growth might increase the quality of carrageenan, including increased gel strength.

SRC spectral analysis

The FTIR spectra analysis showed that all samples had an absorption peak that indicates ester sulphate bond, 3,6-anhydrogalactose, and galactose-4-sulphate (Dewi et al., 2015). Glycosidic linkage was also present at 1029–1034 cm^{-1}. Peak absorbance around 1150 cm^{-1} and 1010–1030 cm^{-1} represents C-O and C-C stretching vibration of the pyranose ring, a typical structure in polysaccharides (Gómez-Ordóñez and Rupérez, 2011). Peak absorbance at approximately 845 cm^{-1} and around 930 cm^{-1} indicates D-galactose-4-sulphate and 3,6-Anhydro-D-galactose, respectively, which is ordinarily present in kappa-type carrageenan (Pereira et al., 2013). A similar peak was observed in the SRC sample (wavenumber 924–925 cm^{-1} and 843–844 cm^{-1}). Other types of carrageenan, such as iota carrageenan, have an additional peak band at around 805 cm^{-1} associated with 3,6-anhydro-D-galactose, usually not present in kappa-carrageenan (Webber et al., 2012). Additional peak band was also present in lambda-carrageenan, which had broadband between 820–830 cm^{-1} indicating high sulphate content (Pereira et al., 2009). The samples in the present study did not have an additional band that was characteristically identical to iota-carrageenan (805 cm^{-1}) and lambda-carrageenan (820–830 cm^{-1}), hence confirms that the SRC contained kappa-carrageenan.

Conclusion

Kappaphycus alvarezii was discovered to be the most farmed seaweed species on Java's northern coast, while *Kappaphycus striatus* was only found grown on Tidung Island in Seribu Islands Regency. In the northern coastal region of Java Island, diverse *Kappaphycus* cultivation produced different degrees of SRC quality, including yield, gel strength, viscosity, and chemical properties. *Kappaphycus alvarezii* cultivated on Pari island produced the highest SRC yield. The highest SRC gel strength value was obtained from *K. alvarezii* from Tlaga village in Karimunjawa island, while Legon village *K. alvarezii* produced the highest viscosity level. The physical and chemical properties of SRC, particularly ash content, moisture, and viscosity, passed the FAO standard, except for the sulphate content, which was lower than the standard threshold limit. The study also found several quality parameters correlated with environmental factors based on linear relationships, e.g., SRC viscosity and gel strength with water pH value, gel strength with salinity, and ash content with depth. Based on comparable FTIR spectra absorption patterns, all SRC samples were identified as containing kappa-carrageenan.

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