A Comparative Study on the Physiochemical Properties of Semi-Refined Carrageenan from Low Industrial Grade Seaweed and High-Grade Seaweed of Kappaphycus Alvarezii

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Abstract. A study on the physiochemical properties of semi-refined carrageenan (SRC) derived from low industrial grade seaweed (LIGS) were conducted and compared to semi-refined carrageenan from high-grade seaweed (HGS) of Kappaphycus Alvarezii and others researches. The morphology of SRC (LIGS and HGS) was investigated through scanning electron microscopy (SEM) and physiochemical properties of SRC through Fourier Transform Infrared (FTIR) analysis, particle size analyser, proximate composition (moisture, ash, acid insoluble ash, acid insoluble matter), sulphate and mineral content by inductively coupled plasma optical emission spectrometry (ICP-OES), colour, pH, viscosity and gel strength. SRC-HGS showed smaller particle size than SRC-LIGS from SEM and particle size analysis. SRC-LIGS exhibited lowest lightest, but highest yellowness, with $L^*$ and $b^*$ values reported 57.77 and 15.9, respectively. The gel strength of SRC-LIGS was lower than SRC-HGS. The SRC-LIGS show a comparable thermal properties, sulphur and mineral content, ash, acid insoluble ash and moisture content from SRC-HGS, which suggest that these SRC-LIGS have good potential as renewable resources materials.

1. Introduction

Seaweed has been utilized for ages as food, in cosmetics, pharmaceutical, fertilizer and nutraceutical [1–3]. The structural cell-wall polysaccharides usually contain an outer amorphous mucilage matrix, which is made up of linear sulphated galactan polymers such as carrageenan, agarans, and alginites as well as the inner rigid component which is cellulose fibril [4,5]. Thus hydrocolloids have been widely used as thickener, stabilizer or emulsifier [2,6]. Kappaphycus Alvarezii a kappa type carrageenan is one of red seaweed species, which is extensively farming in the east Sabah, Malaysia. The production of carrageenan divided into its purification process. There is refined carrageenan (RC) and semi-refined carrageenan (SRC).

However, production of RC is very costly and time consuming due to complex purification process during extraction, whereas SRC is much easier to prepare and cost-effective when compared to RC.
The flourishing seaweed industry by Mini estate system driven by Department of Fishery, Sabah under Entry Point Project (EPP). This project identified seaweed as one of the high-value commodities in the Malaysia National Agro-Food-Policy (2011-2020). In 2013, production has achieved one metric ton per day and is targeted to produce 10 metric tons per day by 2020 [9]. Due to the high demand of RC and SRC in the market, there was an abundant leftover low-grade seaweed after processing thus RC and SRC from high-grade seaweed (HGS). There was no previous report of the production of SRC from low-grade industrial seaweed (LIGS). This study designed to introduce LIGS as a raw material in the production of SRC. The aim of this research to produce SRC from LIGS by alkaline treatment and compare with SRC from high-grade seaweed (HGS) in term of physiochemical and morphology properties.

2. Materials and methods

2.1 Preparation of SRC-LIGS and SRC-HGS

LIGS and HGS from species Kappaphycus Alvarezii obtained from a local supplier at the east coast of Sabah, Malaysia washed thoroughly with water to remove any residual salt, sand or other impurities. The cleaned LIGS and HGS then dried overnight in a laboratory oven at 45°C to remove any excess moisture.

For SRC, the dried LIGS and HGS treated with a hot alkaline solution of potassium hydroxide (KOH) at 70 °C for 30 min with KOH concentration of 6% w/v and seaweed to solution ratio (SSR) was maintain at 1:10. The treated LIGS and HGS was cooled down to room temperature and was washed with water several times to remove any excess KOH until it turned neutral (pH 7) before being dried in an oven (45°C) overnight. The dried SRC-LIGS and SRC-HGS were chopped, ground and sieved before kept in an airtight container to avoid further moisture absorption from the environment until further analysis.

2.2 Physical properties

2.2.1 Particle size analysis. The particle size was determined by using a Sympatec HELOS (Helium-Neon Laser Optical System, Sympatec Inc., Clausthal-Zellerfeld, Germany) laser diffraction instrument combined with RODOS dry particle disperser.

2.2.2 Density test. The density was measured using gas intrusion under a helium gas flow with an AccuPyc II 1340 pycnometer (Georgia, USA). Five sample was analysed at 27°C and the average value was computed.

2.2.3 pH. 1.5 % (w/v) LIGS and SRC solutions were prepared and cooled to room temperature (25 ± 0.5). pH meter (Eutech instruments, Singapore) was then used to measure the pH values of the LIGS or SRC solutions. This procedure was done in triplicate for each sample.

2.2.4 Colour. The surface colour of SRC samples was measured using Konica Minolta Chroma Meters CR-400 (Konica Minolta Sensing, Japan). The colorimeter previously calibrated measuring the colour parameters of standardized white glossy ceramic tiles. The colour was evaluated by means of CIELAB coordinate (L*, a*, and b*) (CIE, 1976). Where L* was whiteness (L*=0) or brightness (L*=100), a* was redness (a*>0) or greenness (a*<0) and b* was yellowness (b*>0) or blueness (b*<0). This procedure was carried out five times for each sample.

2.2.5 Viscosity. The SRC powder (1.5 g) was dissolved in 100 ml hot distilled water (± 90oC) and dispersed with high agitation using mechanical stirrer for 15-20 minutes. Next, the sample was allowed to stabilize in a water bath at 80-90 °C for 15 minutes to eliminate bubbles.
Viscosity was determined with Brookfield DV-II (Middleboro, USA) using spindle no 2 and speed of rotation for the viscometer was set at 50 rpm.

2.2.6 Gel strength. SRC solution was prepared by dissolving 1.5 g of SRC powder with 100 ml of hot distilled water at 90 °C with continuous string using mechanical stirrer for 20-30 min. Then, the sample was allowed to stabilize in a water bath at 80-90 °C for 15 minutes to eliminate bubbles. The viscous sample was poured into three containers with the same size (each of height 25 ml) moulded and left to cool to room temperature for 20-30 minutes before closed the container lid. The sample was stored for 24 h and maintain at room temperature (28°C) prior to analysis. TA-XT plus texture analyser single arm (Stable Micro Systems, England) was used to evaluate the compressibility of SRC product. A load cell of 5 kg was used where the maximum force was ± 5 kg. A fixture and an analytical probe were compressed into the sample with return distance 65 mm at return speed of 10 mm s-1 and contact force was 5g. Each run was replicated three times.

2.2.7 Scanning electron microscopy (SEM). The surface morphology of the SRC was observed under scanning electron microscopy (SEM), model ZEISS SUPRA 35 VP (Oberkochen, Germany) with an acceleration voltage of 5 kV.

2.3 Chemical analysis

2.3.1. Proximate analysis. The moisture content of SRC was determined using air-drying oven method (AOAC 934.01). As for ash, determination was carried out in the muffle furnace at 550 °C (AOAC 930.05). For acid-insoluble ash, hydrochloric acid was used for digestion prior to ash. For acid-insoluble matter, SRC samples were treated with sulphuric acid for an acid-insoluble matter determination.

2.3.2 Sulphur and mineral content determination. Approximately 2 g of dried SRC was weighed and placed in the 50 mL glass tube. About 20 mL concentrated sulphuric acid (H₂SO₄), was then pipetted into the glass tube and the vessel was gently shaken for 5 minutes by benchtop shaker SK-300 (Lab Companion, Minneapolis, USA) until stable with no observed bubbles formed. Next, the sample was left standing at room temperature for 2 hours. The solution was then filtered with glass fibre filter paper (90mm) (Toyo Roshi Kaisha, Ltd, Japan), to separate the leached sample. The concentration of the filtered solution was reduced by 100 times with deionized water and filtered again through an ashless Whatman filter. The solution was diluted according to the elements being analysed and was analysed by inductively coupled plasma optical emission spectrometry (Optima 4300 DV ICP-OES, Perkin Elmer, Sheldon, CT, USA).

2.4 Fourier Transform Infrared (FTIR) analysis

The functional group present in powder SRC were analysed by Fourier Transform Infrared (FTIR) analysis. Infrared spectra in attenuated total reflectance (ATR) accessory sampling device containing a diamond/ZnSe crystal. Spectra were scanned at room temperature over the wave range from 550 cm⁻¹ to 4000 cm⁻¹, with a scan speed of 0.20 cm s⁻¹ and 60 accumulations at a resolution of 4 cm⁻¹ using Perkin Elmer Spectrum One (USA).

3. Results and discussion

3.1 Physical properties

Physical properties of SRC-LIGS and SRC-HGS are tabulated in Table 1. The particle size of SRC-LIGS was slightly larger compared to SRC-HGS. The morphology of SRC particle was analysed using
SEM (Figure 1). SRC-LIGS has a rough and uneven surface, is irregularly shaped (flake-like) and is bigger. The small particle size was observed in almost the whole surface of SRC-LIGS. While SRC-HGS has a rough heterogenic surface, is the crooked and twisted shape and is smaller. This finding is in accordance with the particle size analysis, whereas SRC-LIGS has larger particle size compared to SRC-HGS.

The weight of the material is the crucial factor in the material selection process, depending on its application. Density directly correlates to the weight. Both SRC-LIGS and SRC-HGS have the same density of 1.65 g cm\(^{-3}\). Notwithstanding, the density of seaweed is lower than conventional glass fibre (2.5 g cm\(^{-3}\)) but slightly higher than other natural fibre such as banana fibre (1.35 g cm\(^{-3}\)) and kenaf (1.4 g cm\(^{-3}\)) [10]. The pH of both SRC is above 7 and in the range from 9.86 to 10.07. This finding was in agreement with JECFA (2006) [11], pH of SRC must in the range 8 to 11.

Calorimetry results showed that SRC-LIGS and SRC-HGS have similar value of yellowness \(b^*\) and \(a^*\) value more than 0. Thus indicates both SRC comes from red seaweed family. SRC-LIGS has lower *L value (57.77) compared to SRC-HGS (70.15), indicating that SRC-LIGS was slightly darker than SRC-HGS. This is might be due to the source of LIGS which from a low-grade seaweed in the market. The viscosity of SRC-LIGS was lower compare to SRC-HGS. Thus the high-grade seaweed might influence the viscosity of SRC solution. As the presence of free ions will influence the rate of viscosity [12], the SRC-HGS might have higher free ions that induce helical formation which give higher resistant to a shear [13]. Therefore, SRC-HGS exhibit higher viscosity compared to SRC-LIGS. Gel strength is the amount of force required to rupture the gel [14]. SRC-LIGS has lower gel strength (61.6 g cm\(^{−2}\)) as compared to SRC-HGS (73.2 g cm\(^{−2}\)). This might be due to higher degree aggregation and packing of SRC-HGS compared to SRC-LIGS [15].

Table 1. Physical properties of SRC-LIGS and SRC-HGS.

| Sample | Particle size (µm) | Density (g cm\(^{-3}\)) | pH  | *L   | *a   | *b   | Viscosity (cP) | Gel strength (g/cm\(^{2}\)) |
|--------|-------------------|--------------------------|-----|------|------|------|----------------|-----------------------------|
| SRC-LIGS | 9.64              | 1.65 ± 0.00             | 10.07 ± 0.13 | 57.77 ± 1.63 | +1.76 ± 0.08 | +15.06 ± 0.12 | 50.93          | 61.6 ± 0.41                  |
| SRC-HGS | 5.98              | 1.65 ± 0.00             | 9.86 ± 0.15  | 70.15 ± 2.39  | +1.03 ± 0.27  | +15.9 ± 0.38   | 93.87          | 73.26 ± 6.94                 |

Figure 1. SEM morphology of (a) SRC-LIGS and (b) SRC-HGS particles at 300x magnification.

3.2 Chemical analysis

Seaweeds easily absorb moisture over time due to its hygroscopic properties. Moisture content for both SRC is 7.08 and 6.44 %. Thus met the FAO standard, the maximum moisture content allowed in SRC is 12 %. The international standard of ash levels in SRC is at least 15 %, but not more than 40 % [11]. Both SRC has the ash content meet the FAO specification. The acid insoluble ash (AIA) measurement is created to measure the amount of insoluble ash in diluted hydrochloric acid. The results show that AIA for both SRC has met the international standard, which must less than 1%. Acid-insoluble matter (AIM) carrageenan consists of a network of cellulose that is normally present in seaweed cell walls [7].
AIM for both SRC meet the FAO specification which less than 15%. However, AIM for SRC-LIGS was higher compared to SRC-HGS. This might be due to the higher cellulose content and can be proven by the darker colour of SRC-LIGS than SRC-HGS. The sulphate in SRC is mainly derived from galactans in red algae and located at C-4 of the D-galactopyranose unit [2,16]. The lower sulphate content of SRC-LIGS is due to the lower grade of seaweed. Red seaweed (K.Alvarezii) contains high vitamins and minerals that are important in human nutrition. The mineral composition of K.Alvarezii is affected by the seasonal year, geographic area and water temperature [17,18].

From Table 2, the mineral content of SRC-HGS was higher compared to SRC-LIGS. The ash content paralleled the mineral contents. The ash contains SRC is made up from minerals such as sodium and potassium. Hence, the SRC-HGS with higher mineral content also had the higher ash content (22.97%). Moreover, the mineral content had an extensive effect on the gel strength of SRC. As mention earlier, the gel strength of SRC-HGS was higher due to the degree of aggregation of SRC-HGS molecules. Thus aggregation induced by cation in SRC [13]. Apart from that, the higher mineral content of SRC-HGS also induces higher shear rate as potassium induce the formation of helical formation in SRC [13]. This concurrent with viscosity results which SRC-HGS exhibit higher viscosity.

### Table 2. Chemical composition of SRC-LIGS and SRC-HGS.

| Chemical composition | Moisture content | Ash (g/L) | Acid-insoluble ash (%) | Acid-insoluble matter (%) | Sulphate (g/L) | Sodium, Na | Potassium, K | Calcium, Ca | Magnesium, Mg | Iron, Fe |
|----------------------|------------------|-----------|------------------------|--------------------------|---------------|------------|--------------|-------------|---------------|---------|
| SRC-LIGS             | 7.08 ± 1.15      | 19.39 ± 1.71 | 0.02 ± 0.02            | 10.75 ± 1.05             | 322.1 ± 1.05  | 269.8 ± 2.56 | 3189 ± 91.9  | 605.4 ± 2.1  | 289.3 ± 0.05  | 24.3 ± 0.05 |
| SRC-HGS              | 6.44 ± 1.79      | 22.97 ± 4.42 | 0.19 ± 0.00            | 8.52 ± 0.02              | 388.2 ± 2.0   | 314.3 ± 4.28 | 4175 ± 107.3 | 1491 ± 3.33 | 453.3 ± 0.28  | 61.6 ± 0.28 |

### 3.3 FTIR analysis

The FTIR spectra for SRC-LIGS and SRC-HGS were almost identical (Figure 2). All spectra displayed an absorption band at around 1220 – 1230 cm⁻¹ related to ester sulphate, 1035 to 137 cm⁻¹ represent the glycosidic linkage, 920-930 cm⁻¹ related to 3,6-anhydro-D-galactose and 845 cm⁻¹ represent D-galactose-4-sulphate [19–21]. From this spectrum, it can be confirmed that the source of SRC-LIGS and SRC-HGS came from K.alvarezii species.

![Figure 2. FTIR spectra of SRC-LIGS and SRC-HGS.](image)
4. Conclusion

In this study, SRC from LIGS and HGS from Sabah were produced and the physicochemical properties were investigated and compared. The SRC-LIGS and SRC-HGS showed a significant difference in particle size, colour, particle morphology, viscosity, gel strength and chemical composition. These differences are possibly due to a geographical area, seasonal variation and variation in seaweed grade. Whereas, SRC-LIGS has a larger particle size and darker compared to SRC-HGS. The viscosity and gel strength of SRC-LIGS slightly lower from SRC-HGS. Moreover, the AIM content, sulphur and mineral content of SRC-LIGS was lower compared to SRC-HGS. The AIA content for SRC-LIGS was higher from SRC-HGS. FTIR analysis showed that SRC-LIGS was from the same source as SRC-HGS. The characteristic shown by SRC-LIGS manifest that this seaweed also possesses a great potential as renewable resources in applicable applications in lower cost.

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