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CHARACTERIZATION and QUALITY of SEMI REFINED CARRAGEENAN (SCR) PRODUCTS FROM DIFFERENT COASTAL WATERS BASED ON FOURIER TRANSFORM INFRARED TECHNIQUE

Eko Nurcahya Dewi*, YS. Darmanto and Ambariyanto

Graduate School of Coastal Resources Management
Diponegoro University, Semarang 50275, Central Java, Indonesia

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ABSTRACT

Semi Refined Carrageenan (SRC) product is considerably cheaper and easy to produce as a natural polysaccharide hydrophilic in food and other product. The aim of this research was to evaluate the quality of two different SCR products come from different coastal waters of seaweed culture. The products were then compared to commercial SCR on the quality based on their chemical quality (Fourier Transform Infra Red, sulphate content and heavy metals) and physical quality (gel strength and viscosity). The method of FTIR was useful as a quality screening for commercially seaweed culture at different geografic places based on their chemical structure.

Raw material that have been used for SCR product was Cottonii with k-carrageenan type. FTIR spectroscopy showed the molecular present in three different samples are quite similar, it can be found spectra band of 1257,59 cm^{-1} which referred to esther sulphate, 933,55 cm^{-1} for 3,6 anhydrogalactose and 848,68 cm^{-1} assigned to galactosa-4- sulphate respectively. The SCR product from different coastal waters were different on their quality.

Keywords : SRC, characteristic, quality, coastal water, FTIR

*) **Correspondence**: Phone/Fax: 62-8122810535, e-mail: nurdewisatsmoko@yahoo.com

INTRODUCTION

The industrial manufacture of carrageenan is no longer limited to extraction of pure form carrageenan, but also semi-refined carrageenan *(SCR*). Nowadays, *SCR* is a kind of natural polysacccharide hydrophilic widespread used as an alternative of carrageenan properties. In food and other products, carrageenan works as a thickener, stabilizer, and gelling agent. Because it comes from algae, it can be used as a substitute for gelatin function for vegetarian. Earlier *SRC* is not a food grade, *SCR* mostly applied in the pets food industry and in the dairy industry where clarity is not an important issue. However, recently there has been improved method in producing a suitable food additive for human consumption, such as to simulate homogenity and accelerate the reaction process with seaweed chopped prior to the KOH cooking. Further, the product was also processed by applying high food safety procedure so *SRC* are permitted in human consumption (Philip, 2002).

The *SRC* product is considerably cheaper than refined carrageenan, since there is no involved steps such as centrifugation, filtration and alcohol precipitation that must be recovered. In this process, no distillation equipment to purify alcohol, and also there was no refrigerated process to freeze the gels as in the carrageenan gel pressing extraction processing method. Water and hot alkali are used to wash out residual minerals, soluble protein, and fat leaving. *SRC* always contains residual cellulose with some carrageenan that is still locked into the cellulose cell wall, since actually carrageenan itself not technically extracted out of the seaweeds (Mc Hugh, 2003).

In FDA regulations, *SRC* is now accepted as carrageenan and there is not legal distinction. EU regulations are different : semi refined carrageenan is allowed as its own e number (E407a) under the name Processed Eucheuma Seaweed (*PES)* or *PNG* (Philiphine Natural Grade), and E-407 for carrageenan (Phillips, 2002). The distinction is drawn on the level of residual cellulose in the product. In refined carrageenan most of cellulose has been removed by filtration during the processing, while the *SCR* still contains the cellulose as in the original seaweed. Refined carrageenan will therefore give a clear solution, while *SCR* gives a cloudy solution.

The *SRC* form produce, is obtained by the following processing steps: (1) pre-rinsed raw seaweed; (2) cooked or boiled the seaweed in an aqueous solution of potassium hydroxide with in order to cause desulfation at the 6-position of the galactose units of the carrageenan, to create recurring 3,6 anhydrous galactose polymers by dehydration and reorientation hence carrageenan was not dissolving in the solution; (3) cleaned and washed the seaweed in running water until the waste water seaweed pH becomes neutral ; (4) and the reminded was carrageenan and cellulose, which then dried, chopped and finally grinded to a powder form namely *SRC* or seaweed flour.

Generally, research has been done on carrageenan rheological properties since it may vary widely depend on the algal sources, the method of seaweed culture, age of harvesting, and the processing method.

Molecular interactions or bond in an carrageenan and cellulose molecule ethers could occur due to the presence of polyhydroxy groups in the cellulose backbone which can form intermolecular hydrogen bonding with hydroxyl groups of carrageenan as the carboxyl groups of the latter polysaccharide. FTIR or Fourier Transform Infrared Spectroscopy refers to a fairly recent development in the manner in which the data is collected for only a small sample of material and converted from an interference pattern to a spectrum hence it could be a good confirmatory tool in this respect (Tuvekene *et al.,* 2006).

The FTIR spectrum of the unknown materials can be compared with the spectra that have been identified for establish materials. The widely application of seaweeds phycocolloids (e.g. agar, carrageenan and alginat) are related to their gelling ability, thickening dan stabilizing the product. These seaweed polysaccharides present a specific chemical structure related with the taxonomic position of the algae. To know the quality of seaweed as a raw material for *SCR* products on its chemical compound from different water origin in Indonesia, therefore FTIR is applied.

As a spectroscopic techniques, FTIR has provided accurate identification of the polysacharides natural composition produced by these seaweeds. It is possible to identify the chemical structure of seaweed colloids in ground seaweed samples as in extracted material.

The study was aimed to identify and clarify the chemical structure of *Cottonii* from different coastal waters, it can also be used as a screening for commercially seaweed culture at different coastal waters or place of culture based on their chemical structure.

MATERIALS AND METHODS

Sampling of seaweed Cottonii (K.alvarezii)

Raw seaweed *Cottonii* was harvested, washed with water to remove salt, sand, shells and foreign materials such as epiphyte. All cleaned seaweeds were then sun dried for 2-3 days or until moisture content approximately 35% in order to fullfilled level permitted by food regulation on Indonesian National Standard (SNI).

Semi Refined Carrageenan (SRC) production

Basically, manufacturing process of semi refined carrageenan are as follow : dried seaweed was boiled with temperature where maintained at 80^{o}C in an aqueous solution of potassium hydroxide for 4 hours. The ratio of seaweed and KOH were 1 : 8, based on the dried seaweed weight. At the end of boiling process the wet seaweed was cleaned and washed in a running tap water to have better removal of adhering alkali from the product until the pH of seaweed waste water becomes neutral. After neutralisation, seaweed then chopped, dried, grinded and finally blended to a fine powder that passed through 40 -60 uμ mesh size sieve. Then it was stored in sealed plastic bags at room temperature for further analysis.

Quality Parameter Analysis

- FTIR (Fourier Transform Infrared)

Approximatelly 2 mg dried *SRC* powder were milled with potassium bromide (KBr) to form a very homogen fine powder by using vibrating mill. This powder was compressed into a thin pellet 1 cm in diameter, was then recorded using FT-IR spectrophotometer (Prestige- 21)

Measurement of gel Strength of *SRC*s, Viscosity and Sulfat content

- Gel Strength of *SCR*s were measured by weighing 1,6 % *SCR* powder and diluted in 0,16 % hot aquaeous KCl solution. Gel was allowed to form in the cylinder stainlesteel with 5 cm hight and 4 cm diameter, and then kept in the refrigerator overnight. The gel strength was measured using TA-XT Plus *Texture Analyzer*.
- The viscosities of the solutions (3 g sample of *SRC* in 250 ml aquadest) were then measured at with a Brookfield DV-E viscometer and 18 spindle at atmospheric pressure and temperature of 25 °C.
- Sulfat content was analysed using the method of sulphate hydrolysis followed by precipitation of sulphate as barium sulphate. 1 g sample of *SCR* was refluxed with 50 mL 0,2 N HCl for 6 hours until it becomes colorless solution. Ten mL $BaCl_2$ was added and boiled for 2 hours. After cooled, the $BaSO_4$ precipitation were filtered using ashless paper filt and then burned in a furnace for 1 hour at 1000^0C. The white ash was weighed.

  Sulphate content (%) was calculated is follow =

  P ($BaSO_4$ precipitation) x 100% x 0.4116

- Measurement of heavy metal

Lead : The sample (7,5 gram) was wet-ashed destructed with nitric and perchloric acids and analysed using flame atomic absorption spectrophotometry at 283.3 nm using an acetylene oxidizing flame. A standard curve was plotted at absorbance against µg Pb/ml for the blank and working solutions.

Cadmium : the procedur was proceeded as determination of lead, using 228.8 nm as the analysis wavelength. 5 grams were accurately weighed of a dry powdered test sample and put into a 250 ml erlenmeyer flask. The sample was wet with 5 ml of D/D water and then 10 ml of 1:1 nitric acid-perchloric acid was added. Then gently heated on a hot plate (100-150° C) until all of the dark fumes are evolved and the solution turns yellowish or colourless; swirl the flasks occasionally.

Mercury : 5 grams dry powdered test sample were weighed into a 250 ml Erlenmeyer flask. The sample was wet-ashed with nitric and perchloric acids and analysed using hydride-generation atomic absorption spectrophotometry with a 253.7 nm wave length.

Statistical Analysis

A spectra bands of FTIR was analyzed using a descriptive analyzed, the bands was compared with commercial product *SRC* (Gel strength, viscosity data are already supported on the packaging). Data on gel strength, viscosity and sulfat content were analysed by Anova. Data on heavy metals content are measured triplicates.

RESULTS AND DISCUSSION

FTIR spectroscopy shows the molecular present in three different samples are quite similar **(Fig.1).**

One of characteristic quality product of carrageenan is influenced by algal source. Figure 1 shows the same spectra bands present both in samples from different coastal water origin and the commercial *SRC* . The spectra bands samples at approximately 316 to 3800 (cm^{-1}). Since there was rare spectra bands of *SCR* product previously is described, it is essential therefore the spectra bands of samples are compared to a commercial *SCR*. In *SCR* from Sekotong coastal water, it was be found spectra band of 1257,59 cm^{-1} which referred to ester sulphate, 933,55 cm^{-1} for 3,6 anhydrogalactose and 848,68 cm^{-1} assigned to galactosa-4- sulphate, respectively.

The result was compared to Distantina *et al.* (2010) research on Sigma carrageenan, it was indicated there were similar result with spectra bands present at Sigma commmercial which were 848,68 cm^{-1}, 925,55 cm^{-1} and 1257,59 cm^{-1} respectively. Hosseinzadeh (2009) who grafting acrylamide on to kappa-carrageenan using potassium persulfate as a water soluble oxidizing initiator reported spectra on 1015, and 1222 cm^{-1} can be attributed to D-galactose-4-sulphate, 3,6-anhydro-D-galactose, glycosidic linkage, and ester sulfate stretching of kappa carrageenan, respectively.

The interesting point was found on the materials compound for *SRC* from Sekotong and Mlonggo coastal waters and *SRC* commersial, the spectra bands of materials were significantly similar to that *Sigma* carrageenan commercial product. From this point it can be asumed firstly, the quality of raw materials to produce *SRC* as good quality as refined carrageenan such as Sigma commercial product, secondly these area can be develop as one source of seaweeds raw materials for seaweed extract industry. Thirthly during process of seaweeds extraction, the methods does not induce any significant change in the native of molecular structure in the native seaweed final product.

Van de Velde (2002) studied on carrageenan, reported that usually it has strong, broad band, typicall of all polysaccharides in the 1000 to cm-1 region with 1065 and 1020 cm^{-1} for gelling and non gelling types respectivelly. Wave number (cm^{-1}) : 1220-1260 determinated ester sulphate (since there is linked bound of S=O in the ester sulphate), 1010- 1080 assigned to glycosidic bound, 928-933 : 3,6-anhydrogalactose, 840-850 : galactose-4 sulphate, 825 -830 : galactose-2 sulphate, 810-820 galactose-6-sulphate, 800-805 : 3,6 anhydrogalactose-2 sulphate.

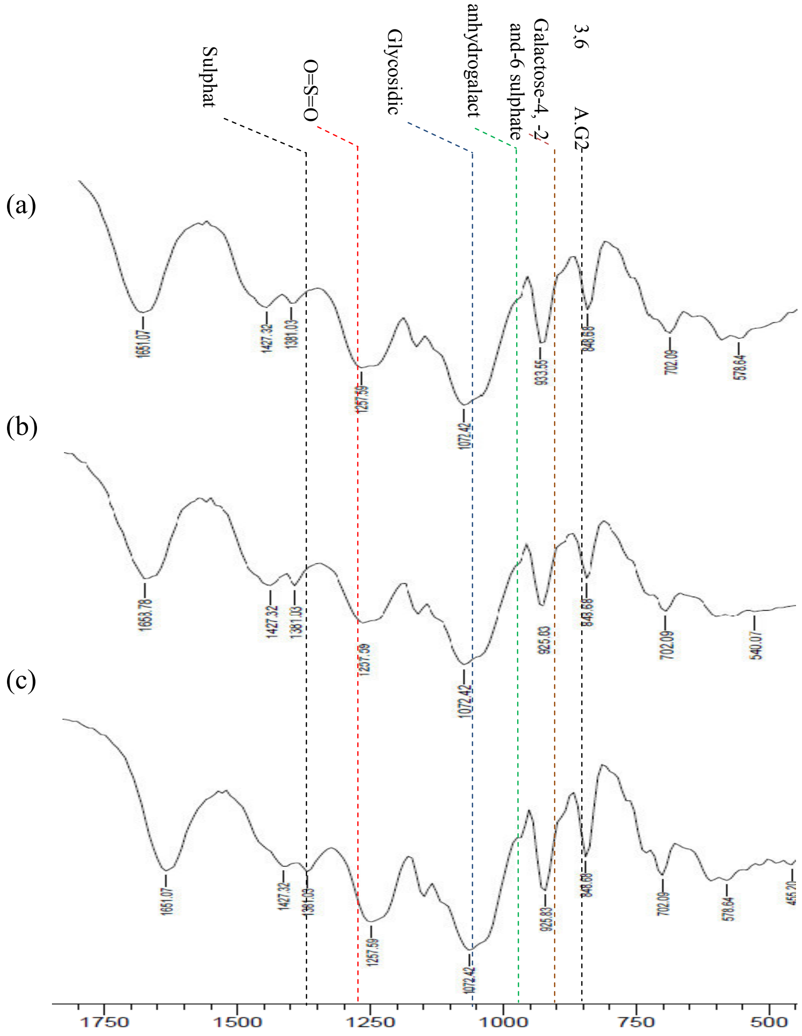

Fig. 1. (a) FTIR of *SRC* seaweed sample from Sekotong, (b) FTIR of *SRC* seaweed sample from Mlonggo (c) FTIR of commercial *SRC*

The main difference of kappa and iota was differentiated from wide spectra appeared, the bands 840-850 cm^{-1} identified for galactose-4-sulphate for kappa (Freile-Pelegrin *and* Robledo ,2006a and 2006b) and the spectra bands of 800-805 (cm^{-1}) which is iota with linked bound of 3,6 anhydrogalactose-2 sulphate (Freile-Pelegrin *and* Robledo, 2007). It is evident that *SRC* product from 2 different coastal waters origin are kappa carrageenaan type, since it extracted from *Cottonii*. Kappa carrageenan produces strong and rigid gels.

Instead of carbohydrates, residu of carrageenan consists of xylose, glucose and uronic acids, other cations such as ammonium, calcium, magnesium, potasium and sodium are present as galactose esther (Van de Velde, 2011).

In addition to this, *SRC* obtained from all the products also contained an additional band at 1072 cm^{-1}, which was indicated as glycosidic bound (Tuvikene *et al*.,2006). It can also be seen another bands of 3448, 72 cm^{-1}, Mecozzi *et al.* (2009), reported that there were an unique peaks at 3400 and 2850 cm^{-1} which is related to interaction between carbohydrate and lipid. The structural of cell wall can also influenced by the heavy metal (Hg, Cu, Cr, Cd) penetration to the cellwall and that is identified from several crosspeaks between 650 and 1750 cm $^{-1}$(Mecozzi*et al.* ,2009). The result was also compared to the spectrum of kappa carrageenan, the IR spectrum of the kappa-carrageenan (kC)-PAM (graft copolymer synthetic part) shows a new characteristic absorption band at 1665 cm-^{1} verifying the formation of kC-g (graffing)-PAM. This peak attributed to C=O stretching in carboxamide functional groups of PAM. The stretching band of –NH overlapped with the –OH stretching band of the kappa carrageenan portion of the copolymer.

One of the key quality attributes in the evaluation of *SRC* product include heavy metal content (Van de Velde, 2002), it is important since it is associated with food safety. Seaweeds have a large biomass in coastal area and are usually eaten by people at that area. Hence it is necessary to clarify of the amount of heavy metals accumulation. Concentration of seaweeds heavy metals showed differences depending on variation or elements or seaweed (Sudharsan *et al.,* 2012).

The structural of cell wall can also influenced by the heavy metal (Hg, Cu, Cr, Cd) penetration to the cellwall and that is identified from several crosspeaks between 650 and 1750 cm^{-1}· The wide band at near 3300 cm^{-1} is also assigned as a carbon bounding material. The accumulation of heavy metals in the red algal affect several biological processes for example as cellular metabolisme and inhibition of photosynthesis. The accumulation of Cd even at very low concentration can cause the physiological disturban like protein, carbohydrate and pigmen concentration.

Table 2. Heavy metal content in SRC samples

Samples	Pb (ppm)	Cd (ppm)	Hg (ppm)
Sekotong water	3,685 ± 081 (2mg/kg)*	0,540± 053 (2mg/kg)*	Not detected (<1 ppm)*
Mlonggo water	8,423 ± 0,45	0,167± 0,55	Not detected
Commercial	9,344± 0,70	0,351± 0,35	Not detected

Note : *value permitted by regulation (Benford *et al.,*2008).

No heavy metal (Hg) is detected in two samples from two different coastal waters and the commercial κ-carrageenan samples, except for lead (Pb) and cadmium. The concentration of lead detected in the kappa(κ) carrageenan samples fell within the acceptable ranges (<2 mg/kg) set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). However the concentration of mercury detected in the κ-carrageenan samples higher than the acceptable ranges (<2 mg/kg) set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Benford *et al*., 2008). The different content of heavy metal, is mainly influenced by the place of seaweed cultivation. Mlonggo water is known as coastal water which have effluent from domestic waste and it is influenced by North Java Sea, whereas Sekotong water is a bay area water which not influenced by domestic waste water and it mostly influenced by Indian Ocean. Qari and Siddiqui (2010) reported that red seaweed has more ability to uptake these such heavy metals (Fe, Mn, Cu, Ni, Zn, Cr and Pb) compared to brown and green seaweed.

The chemical and physical parameter from two different product from different coastal waters are presented on **Table 3**.

Table 3. Quality Parameter of Semi Refined Carrageenan From different Coastal Waters of Seaweed Culture

	Semi Refined Carrageenan		
Parameter	**Sekotong**	**Mlonggo**	**Commercial**
Gel strength (g/cm^2)	257,948 ± 1,292	262,917±1,575	431± 0,000
Viscosity (cPs)	22,703 ± 0,109	20,592± 0,052	25,0 ± 0,000
Sulphate (%)	8,719 ± 0,121	7,600± 0,948	4,513± 0,077

Table 3 shows , that the quality of SRC from two different places of seaweed cultured are lower on quality compared to the commercial *SRC*. The gel strength quality is lower than standard specification which is 400-600 g/cm^2, (Mc Hugh, 2003). The quality different of gel strength is most probably caused by method of processing, temperature and length time of extraction. The gel strength are related with sulphate content, the higher sulfat content the lower gel strength. The sulphate content which is related to gel strength is galactose-6-sulphate. The length time on alkaline solution are used commercially to achieve a high level of precursor conversion to kappa carrageenan.

CONCLUSIONS

From the study conducted, it can be concluded that :

- *Semi refined carrageenan* products from Sekotong and Mlonggo coastal waters belong to kappa carrageenan type.
- FTIR spectroscopy showed the molecular present in three different samples were quite similar, it was band of 1257,59 cm^{-1} which referred to ester sulphate, 933,55 cm^{-1} for 3,6 anhydrogalactose and 848,68 cm^{-1} assigned to galactosa-4- sulphate respectively.
- The quality of commmercial product based on gel strength and viscosity were better than semi refined carrageenan samples from Sekotong and Mlonggo coastal waters.

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