

## Production of semi- refined carrageenan from *Eucheuma cottonii*

S Mustapha\*, H Chandar, Z Z Abidin, R Saghravani and M Y Harun

Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM  
Serdang, Selangor, Malaysia

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This study presents alkaline extraction of red seaweed, *Eucheuma cottonii*, to yield semi-refined carrageenan (SRC) of kappa type. Temperatures 60-80°C were suitable for extraction of SRC. At 80°C using 1.0 M KOH, produced SRC contained the highest purity of 3, 6-anhydrogalactose, a lower heavy metal concentration and gave highest rupture force.

**Keywords:** *Eucheuma cottonii*, Extraction, Potassium hydroxide, Semi-refined carrageenan (SRC)

### Introduction

Carrageenan, the third most fundamental hydrocolloid in the world after starch and gelatine, occurs as matrix material in various species of red seaweeds, *Rhodophyta*<sup>1</sup>. It is used as a food additive, stabilizer and viscosifier<sup>2,3</sup>. Main species responsible for most of the current carrageenan production are *Chondrus crispus* (North Atlantic), *Gigartina stellata* (France), and *Eucheuma cottonii* & *Eucheuma spinosum* (Asia). Global market is currently based on three types of carrageenans<sup>4</sup> (kappa carrageenan, lambda carrageenan and iota carrageenan). Kappa carrageenan forms strong, rigid gels when combined with potassium ions in the mixture<sup>3,5</sup>. Iota carrageenan forms weak, elastic gels, while lambda carrageenan is of non-gelling type, forming thick viscous solutions<sup>6</sup>. Production of semi-refined carrageenan (SRC), a cheaper alternative, is relatively new<sup>7,8</sup> in Malaysia. This study determines effect of temperature, alkali concentration and type of extracting agent used for production of kappa carrageenan from *E. cottonii*. Physico-chemical characteristics of gel forming SRC were also found out.

### Experimental Section

#### Alkaline Extraction of Seaweed

Seaweeds of *E. cottonii* (Fig. 1) type were obtained from fishing villages in Sabah state, Malaysia. Seaweed

was pretreated by removing any visible foreign matter and then washed under running tap water for 1-2 min to reduce salt content. Initial weight of carrageenan was recorded prior to sun-drying process<sup>9</sup>. Then, weight of carrageenan was recorded and change in moisture content was calculated. Seaweed was dried until a constant weight was obtained, approx. 40% of its original weight.

Pretreated seaweed was extracted using three different concentrations (0.01, 0.1 and 1.0 M) of two selected alkaline reagents, KOH and Ca(OH)<sub>2</sub>, at different temperatures (50, 60, 70 and 80°C) for 1 h. There were all together 24 samples of pretreated seaweed according to pretreatment method (Table 1). Seaweed (5 g) was extracted with extracting agents (500 ml) based on earlier experiments done on *E. isiforme* extraction<sup>10,11</sup>. This ratio was maintained for every extraction process. Extraction was performed at the specified temperature using water bath as a heating medium. Physical changes were recorded by observing extracting agent and condition of seaweed during and after extraction.

For neutralization, seaweed was soaked in water overnight to reduce pH effectively. Seaweed was weighed again after neutralization. Neutralized seaweed was dried in an oven at 105°C overnight, resulting in a 20-25% weight loss of seaweed. Dried seaweed (semi-refined carrageenan or SRC) was then milled, grounded, tested and evaluated to determine presence and quality of carrageenan content.

\*Author for correspondence  
E-mail: saari@eng.upm.edu.my



Fig. 1—*Eucheuma cottoni* obtained from Sabah, Malaysia

### Gel Formation Test

SRC (2 g) was mixed with distilled water (100 ml). Mixture was heated in a water bath at 80°C with continuous stirring until SRC solid became fully dissolved. Distilled water was used to replace any water loss during heating. Solution was then allowed to cool to room temperature (RT). Viscous solution (50 ml) was removed and potassium chloride (200 mg) was added to remaining viscous solution to improve gel properties of sample. Solution was reheated and left to cool<sup>12</sup>. The same procedure was applied for remaining samples. Gel forming solutions was then stored in refrigerator at 5°C.

### SRC Properties Analysis

FTIR spectrometer (Perkin Elmer Spectrum 2000, USA) was used to analyze SRC sample (wavelength region, 650-1400 cm<sup>-1</sup>). A background spectrum containing no SRC was run prior to sample analysis. Functional group identification was based on combined compendium of food additive specifications as per JECFA<sup>12</sup>. Gel strength was tested using texture analyzer (TA-Xt Plus, Stable Microsystems, UK) in compression mode. Standard method for testing gel strength was adapted from British Standard Method for Sampling and testing gelatine (BS 757:1975). All cooked gels were compressed for a distance of 10 mm using a cylindrical probe (20 mm diameter aluminium) at a speed of 1 mm/s. During this period, changes in the applied force were recorded to obtain the texture profile. Heavy metal analysis in SRC samples was done using ICP-OES machine (Optima 7300 DV, Perkin Elmer). Samples were first digested using conc. H<sub>2</sub>SO<sub>4</sub> (R&M Chemicals) for 2 h. The solution was then filtered, leaving behind leached

Table 1—Pretreated seaweed samples

Sample	Reagent concentration M	Temperatures °C
A1,2 <sup>a</sup>	0.01	50
B1,2	0.01	60
C1,2	0.01	70
D1,2	0.01	80
E1,2	0.10	50
F1,2	0.10	60
G1,2	0.10	70
H1,2	0.10	80
I1,2	1.00	50
J1,2	1.00	60
K1,2	1.00	70
L1,2	1.00	80

<sup>a</sup>Denotes the type of reagent used; 1 is KOH, 2 is Ca(OH)<sub>2</sub>

samples. Filtered solution was diluted 100 times prior to ICP analysis.

## Results and Discussion

### Gel Formation

#### Effect of Concentration

Seaweed extracted at higher concentrations of extracting agents (> 0.1 M) resulted on gel formation. At low concentration (≤ 0.01 M), solution has lower viscosities compared to those obtained using reagent at high concentration and no gel formation was observed. This is because SRC extraction requires high alkaline conditions for extraction process since carrageenan degrades under hot acidic conditions. During extraction process, KOH solution changed color from colorless to yellow within ½ h of extraction for concentration of 1.0 M at 60-80°C. As concentration of extracting agent was reduced to 0.1 M, color change was observed only after ½ h of extraction. For 0.01 M, only a slight color change was noted after extraction due to contamination of KOH solution in extraction by presence of pigments in carrageenan<sup>13</sup>. For seaweed cooked at higher concentration (≥ 0.1 M), dark yellow and hard seaweed was formed. After it was dried and pounded, brownish SRC was formed. For seaweed cooked at lower concentration (≤ 0.01 M), seaweed formed was soft, spongy and whitish. This indicates that seaweed has been disintegrated and resulted in the loss of carrageenan during rinsing and neutralization processes.

#### Effect of Temperature

Gel formation occurred at higher temperatures (60-80°C) and at 50°C, no gel was observed because

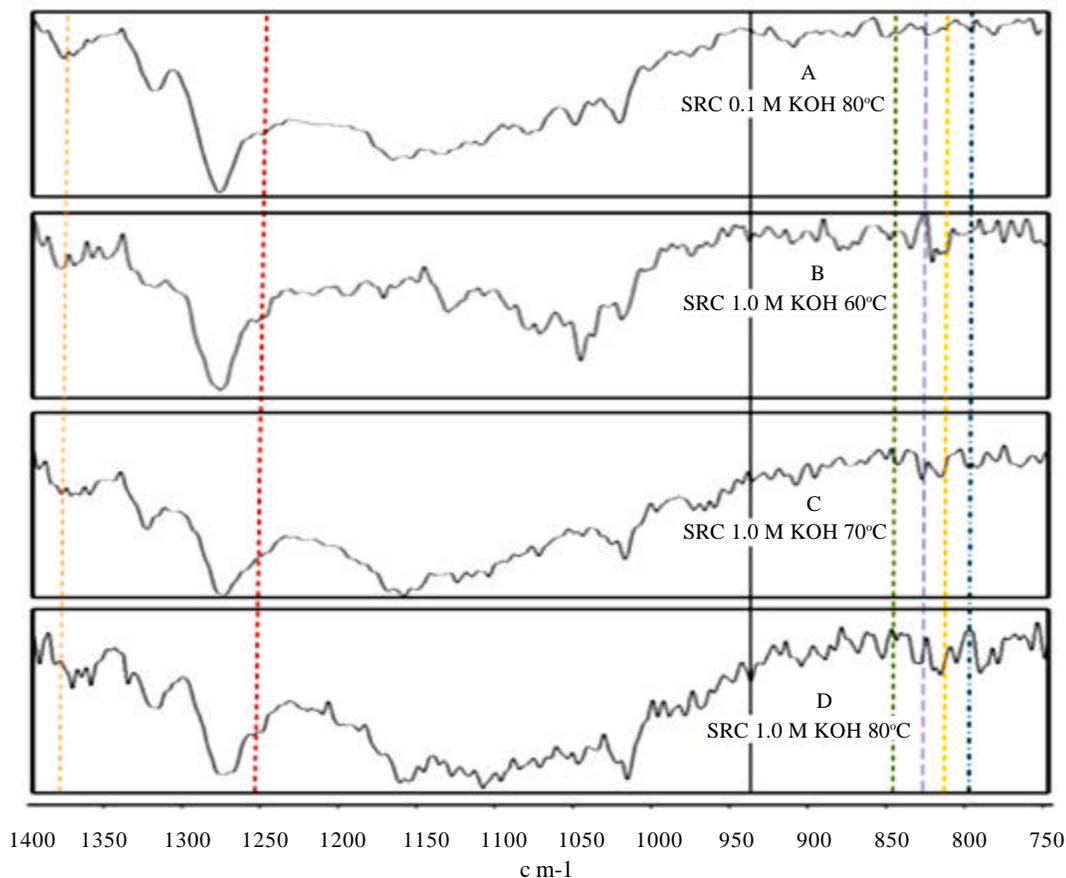


Fig. 2—FTIR spectra of SRC samples: A- No gel formed; B, C, D – Gel formed with increasing tendency with temperature

the chemical process to produce SRC did not take place very well, thus leaving seaweed uncooked. For seaweed cooked at increased temperatures, alkali solution had changed from colorless to dark yellow at increasing rate. SRC obtained was lighter brown, hard and cooked. Prior to addition of KCl, the solutions were generally a cloudy viscous suspension. When KCl was added, gelation of solution occurred, forming a yellowish and slightly opaque gel because of cellulose in SRC<sup>13</sup>. Addition of K<sup>+</sup> induced structural change from coil (disordered) to helix (ordered) conformational transition, followed by subsequent aggregation and network formation between ordered helices in carrageenan solution<sup>14-16</sup>. This process improves gel formation and strength through stabilization of helical conformation of SRC by shielding charge of sulfate groups, followed by ion-dipole binding with polysaccharide into aggregates<sup>17</sup>.

#### Effect of Extraction Agent

Gel formation only occurred when seaweed is cooked with KOH. Seaweed cooked with Ca(OH)<sub>2</sub> could not

form gel in all test conditions. For seaweed cooked with KOH, OH<sup>-</sup> part of the reagent penetrates into seaweed and removes some of the sulphate groups in carrageenan through desulfation at 6-position of galactose unit of carrageenan to create recurring 3, 6 anhydro-D-galactose (3, 6 AG) by dehydration and reorientation, hence increasing gel strength of carrageenan in seaweed<sup>12</sup>. As for the K<sup>+</sup> part of reagent, it combined with removed sulphate group, SO<sub>4</sub><sup>2-</sup> to form potassium sulphate, K<sub>2</sub>SO<sub>4</sub>.

#### SRC Properties Analysis

##### SRC Identification by FTIR

FTIR results of SRC samples (Fig. 2) shows spectra at an absorption band of 1000-1200 cm<sup>-1</sup>, which is related to sulphation level. *E. cottonii* seaweed produced kappa carrageenan and major blocks for this carrageenan<sup>18</sup> are galactose-4-sulfate [G4S; 848 cm<sup>-1</sup> (green dotted line)] and 3, 6 anhydro-D-galactose [3, 6 AG; 933 cm<sup>-1</sup> (black line)]. Peaks for 3, 6 AG are more evident and sharp for SRC samples at higher temperatures and concentration (Fig. 2D). Precursor of 3, 6 AG, galactose-

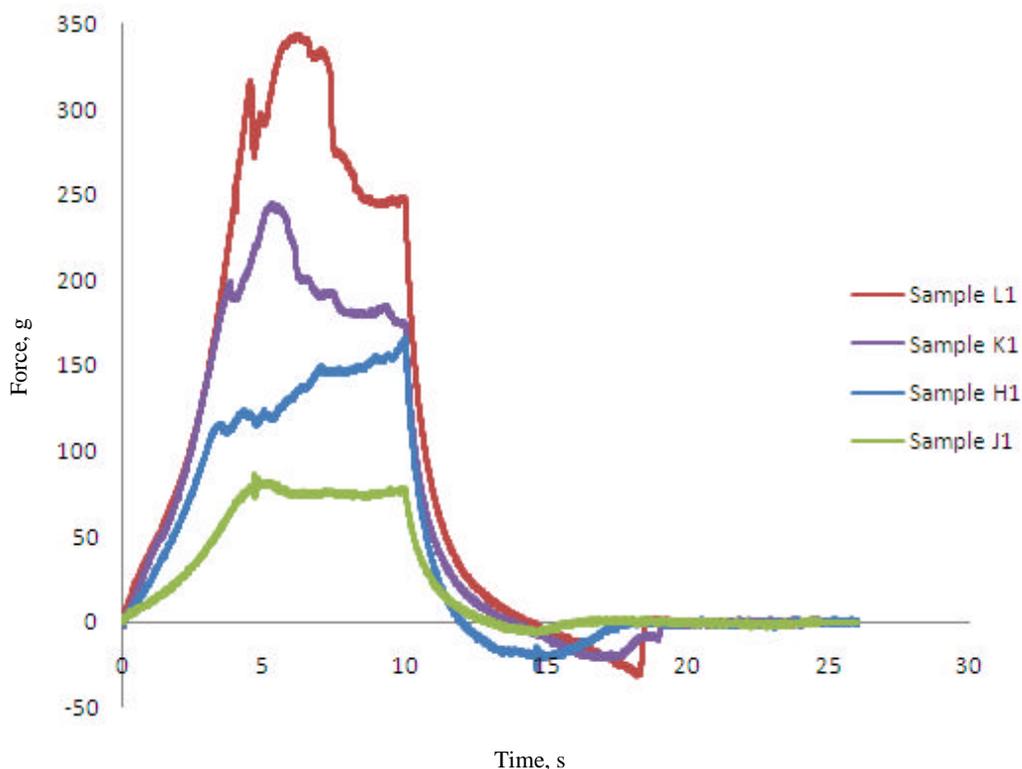


Fig. 3—Textural profile analysis for SRC samples

6-sulfate (G6S) was shown at  $811\text{ cm}^{-1}$  (yellow dotted line). No peaks of other major building blocks [3, 6 anhydro-D-galactose-2-sulfate (3, 6 AG2S) and galactose-2-sulfate (G2S)], which corresponds to iota and lambda-carrageenan<sup>18</sup> respectively, were seen in spectra. For 3, 6 AG2S, no peaks were recorded for spectrum A and D while broad peaks were observed for spectrum B and C. As for G2S, no peaks were recorded for all spectrums. This is clearly demonstrated for SRC extracted in 1.0 M KOH solution at  $80^{\circ}\text{C}$ . Hence, it is confirmed that the type of SRC produced is kappa carrageenan SRC.

#### Gel Strength Analysis

Among gel strength<sup>19,20</sup> (fracturability & hardness force) properties, gel from sample L1 (black line) recorded the largest fracturability and rupture force (Fig. 3), followed by gel from sample K1, H1 and J1. Gel formation involves formation of a 3-dimensional matrix and allows immobilization of water within gel structure. Hermansson<sup>21</sup> stated that it is influenced by the type and quantity of counter-ions in gel solution. However, since

fixed quantity of KCl was used, thus this cannot be used to explain difference in gel strength for different samples. Composition of polysaccharide in SRC<sup>22</sup> influences gel strength. Sample L1, which contains high amount of 3,6 AG in SRC, recorded a larger rupture force compared to sample K1, H1 and J1, due to higher KOH concentrations refer to a greater amount of  $\text{K}^+$  and  $\text{OH}^-$  ions.  $\text{OH}^-$  ions can replace sulphate groups to form more 3, 6 AG. It is reported that high 3, 6 AG content increases gel strength<sup>22</sup>. Cooking seaweed at higher temperatures also increases rupture force. Higher temperature process condition increases reaction rate to form 3, 6 AG. Gels formed at extraction temperatures above  $70^{\circ}\text{C}$  were firm and brittle, could be due to aggregation occurring simultaneously with conformational transitions of carrageenan structure during gel formation<sup>23</sup>. Formation of new and substantial network structures is reported to occur within gel at higher extracting temperatures<sup>24</sup>.

#### Heavy Metals Analysis

Heavy metals analysis shows a huge difference between raw material, *E. cottonii* (sample E) and

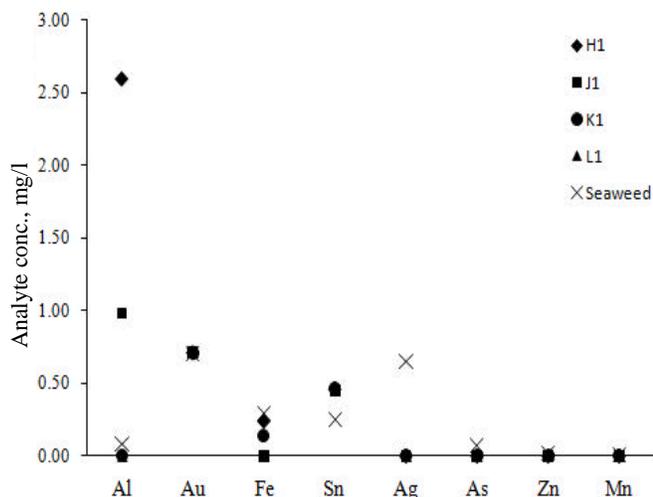


Fig. 4—Analyte concentrations for SRC samples: A-SRC 0.1 M KOH, 80<sup>o</sup>C; B-SRC 1.0 M KOH, 60<sup>o</sup>C; C-SRC 1.0 M KOH, 70<sup>o</sup>C; D-SRC 1.0 M KOH, 80<sup>o</sup>C; E-Seaweed

remaining samples (Fig. 4). Sample E contained additional elements (ferum, silver, arsenic, zinc, manganese and stanum), none of which was detected in other SRC samples except stanum and ferum, may be due to processing methods, as SRC samples had undergone alkaline extraction. These additional elements could have been removed from seaweed cell wall by hot KOH solution<sup>25</sup>. Besides, seaweeds had also undergone neutralization overnight to reduce pH and prevent any toxic accumulation in seaweed. However, a sharp rise in stanum concentration for SRC samples was observed compared to raw seaweed.

Among SRC samples analyzed, sample A (SRC extracted at 80<sup>o</sup>C in 0.1M KOH) has largest amount of heavy metals detected and it recorded a very high concentration of aluminium and ferum, may be due to lower alkaline concentrations used in extraction. Sample D (SRC extracted at 80<sup>o</sup>C in 1.0 M KOH) meanwhile has least concentration of heavy metals followed by sample C (SRC extracted at 70<sup>o</sup>C in 1.0 M) and sample B (SRC extracted at 60<sup>o</sup>C in 1.0 M KOH). All samples (B, C and D) contain aurium and stanum. Concentrations for aurium were consistent for samples (0.71 mg/l). Concentration of stanum was also consistent for samples as their difference between samples were 0.01 mg/l or 2.22%. In addition, Sample C and B contain an additional ferum (0.14 mg/l) and aluminium (0.98 mg/l), respectively, could be due to seaweed being cooked in high concentrations of alkali at high temperatures. Alkali does not degrade cell wall. However, use of high temperature

in combination to alkali degradation in cooking seaweed assists to remove heavy metals and produce intended chemical reactions in seaweed<sup>9</sup>.

## Conclusions

Seaweed extraction at high alkaline concentration and temperature favoured production of SRC. At intermediate concentrations (0.1 M), SRC can only be produced if seaweed is cooked at higher temperatures (> 80<sup>o</sup>C). KOH is a better choice compared to Ca(OH)<sub>2</sub> as it has ability to react with carrageenan in seaweed. Seaweed cooked in 1.0 M KOH solution at 80<sup>o</sup>C was found to be the most optimum. This selection was justified as this sample recorded most intense and sharp peaks of 3,6 AG and G4S in FTIR analysis. Gel formed from this sample also recorded highest values of fracture force and rupture force. SRC samples of 1.0 M KOH at 80<sup>o</sup>C recorded least analyte concentration.

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