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SEASONAL FLUCTUATIONS IN CARRAGEENAN YIELD IN *Eucheuma spinosum* (*Eucheuma denticulatum*) CULTURAL IN COASTAL AREAS OF PULAU BALAMBANGAN, KUDAT, SABAH

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ABSRACT. *Red marine macroalgae of the genus Eucheuma were introduced from the Philippines to Sabah in the late '70s. Their cultivation is of growing importance in Semporna and new culture areas are being introduced with Eucheuma spinosum. To assess the quality and quantity of carrageenan produced by this alga; this study was designed to investigate seasonal fluctuations in carrageenan yield, viscosity and gel strength in Eucheuma spinosum cultured at Pulau Balambangan, Kudat. Carrageenan extracted in alkaline and non-alkaline solvent system produced iota-carrageenan and nu-carrageenan, respectively. Structural information was obtained based on infrared spectrum taken using Fourier Transform Infrared Spectrophotometer. It became apparent that structural differences between iota- and nu-carrageenan were due to the presence of an ether-bridge between C3/C6 of ±-galactose for iota-carrageenan. Data obtained from this study indicated good carrageenan yield in the month of July and December at 56% and 53%, respectively. Carrageenan quality was evaluated based on its viscosity and gel strength. Best viscosity was recorded in Jun/July at of 250~254 pcs while best gel strength was approximately 290 g cm^{-2} at 3% solution in November. Results from this study were also compared with data of the same variety cultured in Danajon Reef, Philipinnes. Based on comparative data analysis, it was apparent that E.spinosum cultured in Teluk Lung, Pulau Balambangan produced iota-carrageenan of consistently high quality and quantity in Jun/July and Dec/Jan.*

KEYWORDS. *Eucheuma spinosum*, *iota*-carrageenan, *nu*-carrageenan, viscosity, gel strength

INTRODUCTION

Macroalgae division Rhodophyta are known to be prolific producers of phycocolloids which have high economic value (Chapman, 1970, Clayton & King, 1990, Castro & Huber, 1997). Phycocolloids such as agar-agar and carrageenan are commercially extracted from red seaweed and their trade accounts for almost US$500 million annually. Carrageenan is extracted from *Eucheuma cottonii* (*Kappaphycus alavarezii* Doty) and *Eucheuma spinosum* (*Eucheuma denticulatum* (Burman) Collins *et* Harvey), carrageenan from the former species is known as *kappa*-carrageenan while *iota*-carrageenan is extracted from the latter. Malaysian waters, especially Sabah's coastal marine areas are ideal for cultivation of these seaweeds and since Doty's report in 1978; many farms were open in Sabah. Hence, *Eucheuma* has shown to be a promising species

for commercial exploitation and has been successfully cultivated on a large scale for the purpose of extracting carrageenan. Many seaweed farms in Sabah are cultivating *Eucheuma cottonii* which yields *kappa*-carrageenan. The main global producers of carrageenan are Phillipines and Indonesia, while Malaysia follows with a production of 3,000 MT per annum. Sabah has about 100,000 acres of suitable coastal waters for seaweed mariculture but only 1,000 acres are presently being utilized. Hence, with such an abundance of resources, it is obvious that seaweed culture could be further expanded in Malaysia particularly Sabah.

Equipped with this vision, Borneo Marine Research Institute of Universiti Malaysia Sabah in collaboration with Lembaga Kemajuan Ikan Malaysia (LKIM) and Kudat District Office embarked on a project targeted to alleviate poverty among the coastal hardcore poor communities of Kudat district by introducing seaweed cultivation. This project started in 1998 and is funded by the Ministry of Rural Development Malaysia. Seaweed culture has been identified as a means of helping these hardcore poor communities to earn extra income and sustain themselves. Both, *E. cottonii* and *E. spinosum*, have been introduced as cultivation crops. Market demand for carrageenan is dictated by the supply of *E. cottonii* which is known to produce *kappa*-carrageenan. In contrast, relatively few seaweed farmers are cultivating *E. spinosum* in Sabah. Initiatives are being taken to better understand the physiology, growth and carrageenan yield of *E. spinosum*, with the intention of expanding culture of this species.

As part of our research effort to gather baseline data for the development of a commercial scale seaweed culture of *E. spinosum* in Sabah, this paper presents the results of qualitative and quantitative analysis of carrageenan content in *E. spinosum* for one year cycle. Comparative analysis of carrageenan extracted from this species with the ones cultured in Phillipines will be made based on type of carrageenan, percentage of yield and physical qualities. It is our hope that the data obtained from this study will enhance our understanding of the quality of carrageenan produced by *E. spinosum* cultured at this site and enable us to forecast any future prospects of its large scale culture in Sabah.

MATERIALS AND METHOD

Collection of samples

Seaweed samples weighing approximately 2 kg were collected monthly (January ~ December 2002) from culture beds at Teluk Lung, Pulau Balambangan, Kudat. These were cleaned of foreign matters and washed in many changes of seawater. It was then immediately transported to lab in polystyrene bags under cool condition. At the laboratory, seaweeds were again washed several times with seawater before there were rinsed with distilled water. Seaweeds were then air dried under shade before extraction of carrageenan.

Carrageenan extraction

Carrageenan was extracted using two extraction methods; 1) Alkaline extraction, and 2) Non-alkaline method. Air dried seaweed samples used for extraction were further dried in oven for 1 – 4 hours to obtain constant weight. 10 g of the dried seaweed was chopped into small pieces and used for extraction.

Method 1. 10g of chopped seaweed was extracted in 500 ml of 0.2M NaOH solution by boiling it for 4 hours. Hot extract was filtered using double layered cheese cloth. Carrageenan was precipitated by slow addition, with stirring, of 200 ml iso-propyl-alcohol. The bulky, white, somewhat gummy precipitate was allowed to settle and collected by low-speed centrifugation. Precipitated carrageenan was washed twice with distilled water decanted, bleached with 95% ethanol. Washing was repeated two more times and the resulting product was dried in oven (60 °C, 6 hr) and then left overnight in vacuo over P_2O_5. Total weight of carrageenan extracted was recorded and calculated as percentage of weight of dried seaweed used.

Method 2. 10 g of chopped seaweed was extracted in 500 ml of distilled water by boiling it for 4 hours. Hot carrageenan solution was filtered using double layered cheese cloth. Carrageenan was precipitated by slow addition, of 200 ml iso-propyl-alcohol while stiring. Precipitated carrageenan was allowed to settle and was collected by low-speed centrifugation (5000 g, 30 mins), washed twice with distilled water and bleached with 95% ethanol. Finally, the product was again washed twice with distilled water before it is dried in oven and then overnight over P_2O_5. Total weight of carrageenan extracted was recorded and calculated as percentage of weight of dried seaweed used.

Carrageenan identification

Films for infrared analysis were obtained by drying 1 ml of 1% of aqueous solution of carrageenan on a smooth surface at 60 °C. The films were then carefully peeled, desiccated in vacuo over P_2O_5, mounted on detachable windows and spectra were recorded on a Nicolet spectrophotometer. This method was used for carrageenan extracted using method 1, method 2 and standard *iota*-carrageenan.

Physical parameters of carrageenan

Viscosity

Reproducible measurements on dilute solutions of carrageenan were obtained with Canon-Fenske viscometer. It provided a means of measuring internal friction of liquids and required the use of dilute solutions. All glasswares were acid-cleaned, thoroughly rinsed, dried and protected from dust. Solutions were prepared with deaerated distilled water and filtered through fine porosity glass filters or membranes (0.45 ¼m) before measurements taken. Detailed measurements were performed according to Hellebust and Craigie (1978).

Gel strength

Gel strength is an important parameter used in determining the quality of gel produced by a seaweed variety. Method used in this study to test gel strength was modified using a 3% solution to enable comparative analysis with carrageenan gel strength as suggested by Corrales and Sa'a (1990).

RESULTS AND DISCUSSION

Figure 1 shows distinct FTIR (Fourier Transform Infrared) spectrums of commercially available *iota*-carrageenan, carrageenan extracted in alkaline solution (Method 1) and carrageenan extracted in non-alkaline solution (Method 2).

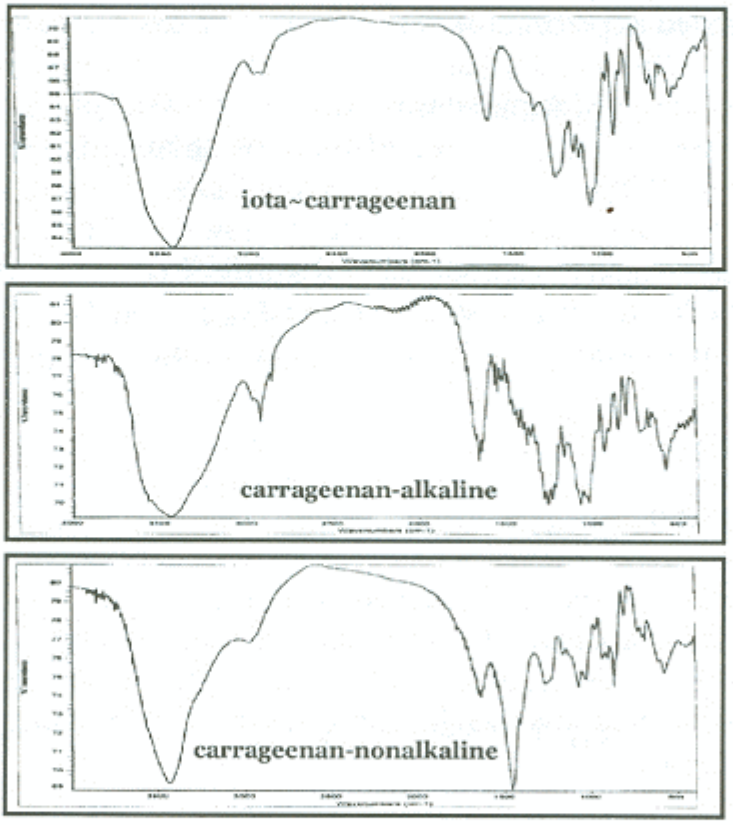
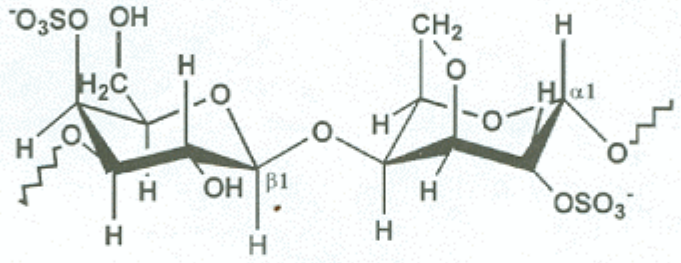


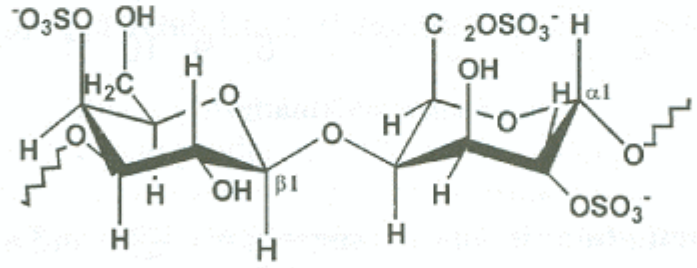
Figure 1. Infrared spectra of carrageenan from *Eucheuma spinosum*, extracted using alkaline (*iota*-carrageenan) and non-alkaline (*nu*-carrageenan) solvent systems.

Spectrum of carrageenan extracted in alkaline solution was comparable with that of the commercially available *iota*-carrageenan while carrageenan extracted in non-alkaline solution showed obvious differences. Infrared spectra of carrageenans are very useful in comparative studies of carrageenan types and their sources (Anderson *et al.*, 1968, Corrae-Diaz *et al.*, 1990). Commercial *iota*-carrageenans are linear polysaccharides made up of alternating ²-1,3-linked D-galactose-4-sulphate and ±-1,4-linked 3, 6 anhydro-D-galactose-2-sulphate residues.

Carrageenans are classified according to the position and content of substitutions on their repeating disaccharide units (Greer and Yaphe, 1984). Carrageenan obtained by alkaline extraction gave ±-galactose with an ether bridge between C-3 and C-6 (C-O stretching). This was evident with the presence of infrared absorbance at 1020 cm^{-1}, 1080 cm^{-1}, 1220 cm^{-1} and 1270 cm^{-1} similar to the commercial *iota*-carrageenan. A broad absorbance in the region of 3400 cm^{-1} can be attributed to O-H stretching of hydroxyl functional groups at C-2 and C-6 of ²-galactose unit. Therefore, it is evident that carrageenan obtained by alkaline extraction has a linear polysaccharide structure made up of alternating ²-1,3-linked D-galactose-4-sulphate and ±-1,4-linked 3, 6 anhydro-D-galactose-2-sulphate as shown in Figure 2. Non-alkaline extraction gave carrageenan void of an ether bridge between C-3 and C-6 of ±-galactose. This was confirmed with the absence of a pair of broad absorbance in the region of 1020 cm^{-1} ~ 1270 cm^{-1}. Carrageenan obtained from non-alkaline extraction is known as *nu*-carrageenan (Å-carrageenan) and is known to be an analogous precursor of iota-carrageenan (Figure 2) (Chapman, 1970).



ι-carrageenan



υ-carrageenan

Figure 2. Repeating disaccharide structures of carrageenan extracted using alkaline (*iota*-carrageenan) and non-alkaline (*nu*-carrageenan) solvent systems.

Figure 3 shows annual fluctuation in carrageenan yield in *E. spinosum* cultured at Teluk Lung, Pulau Balambangan, Kudat. As delineated in Figure 3, annual yield shows a capacious difference between percentage of carrageenan obtained *via* extraction Method 1 and Method 2. It is also a cogent fact that Method 1 gave higher carrageenan yield as compared to Method 2 and could probably be attributed to the presence of ether-bridge in *iota*-carrageenan but not in *nu*-carrageenan. Presence of ether-bridge could help to trap smaller carrageenan chains and reduce their dissolvability. Annual carrageenan fluctuations in *E. spinosum* cultured in Kudat shows a distinct pattern with two peaks, one in July and other in December. Highest yield of *iota*-carrageenan was obtained in July with a maximum of 56%, followed by a gradual decrease to a minimum of

36% in October before renewed increase to the second peak of 53% in December. This value then decreased rapidly to a minimum of 35% before starting on a gradual increase till July. Yield of *nu*-carrageenan also depicted a similar pattern but with a maximum of 45% and 42% in July and December, respectively. In 1990, similar study was carried out for *E. spinosum* cultured in Danajon Reef, Phillipines for 6 months (June to November). Quantitative carrageenan study showed a maximum of 52% and minimum of 21% with no consistent pattern within the study duration (Corrales & Sa'a, 1990). Although the maximum carrageenan content was comparable to the Malaysian variety but the minimum carrageenan content was too low with drastic fluctuations and inconsistency in carrageenan yield between the months during the study period.

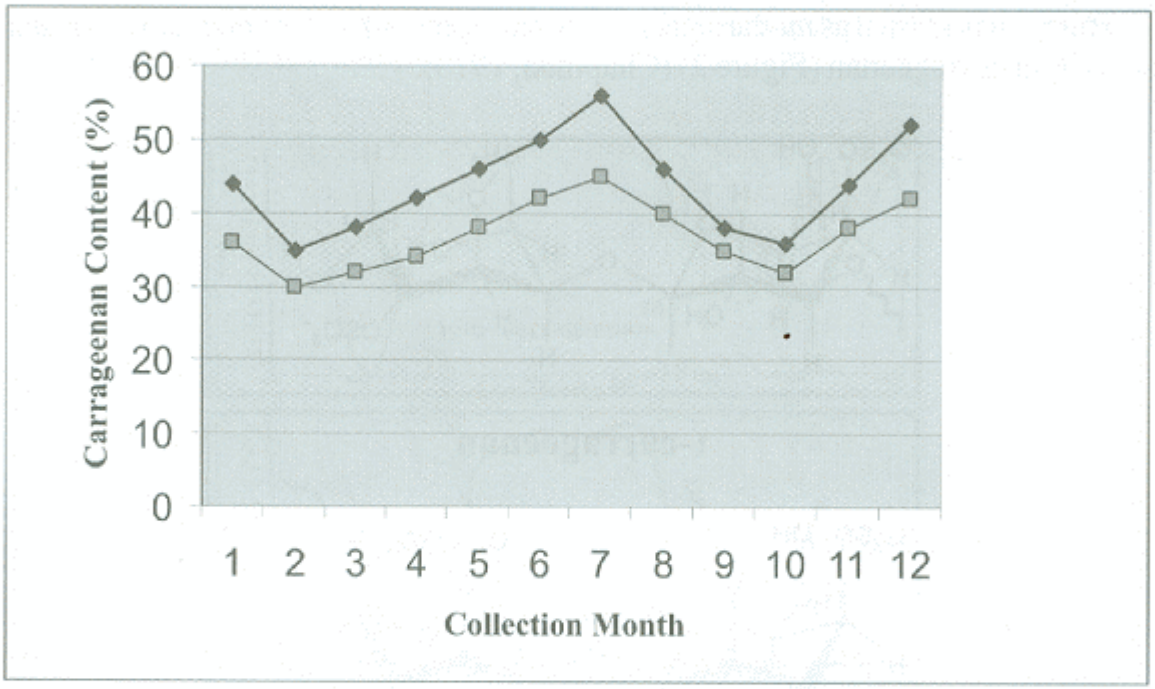


Figure 3. Seasonal fluctuations in *iota*-carrageenan (▢) and *nu*-carrageenan (◆) extracted from *E. spinosum* cultured at Teluk Lung, Pulau Balambangan, Kudat.

Quality of extracted carrageenan was determined based on its viscosity and gel strength of *iota*-carrageenan since *nu*-carrageenan is its only precursor (Chapman, 1970). Figure 4 shows viscosity of carrageenan for every month of sampling. There is a clear pattern in viscosity fluctuation resembling that of annual carrageenan yield. Highest viscosity was about 254 pcs in June and followed by 210 pcs in January as its second peak. Lowest viscosity was seen in April and November at about 158 pcs, respectively. Comparatively, viscosity level of *E. spinosum* from Danajon Reef was inconsistent throughout the study duration with a maximum of 170 pcs in June and a minimum of 47 pcs in October. Gel strength measurements were only carried out for samples taken in January, April, June, and November. Selection of samples was based upon their viscosity; i.e. highest viscosity and lowest viscosity. Best gel strength was 290 g cm^{-2} at 3% solution seen in sample collected in November, while samples taken during other months showed fluctuations in the range of 120 ~ 140 g cm^{-2} at 3% solution. Gel strength values obtained from this

study are comparable to the findings reported for *Eucheuma* from Danajon Reef, Philippines. Seasonal differences in carrageenan yield and quality could be attributed to changes of abiotic factors of seawater and weather at seaweed culture site as reported by Johnstone and Olafsson (1995).

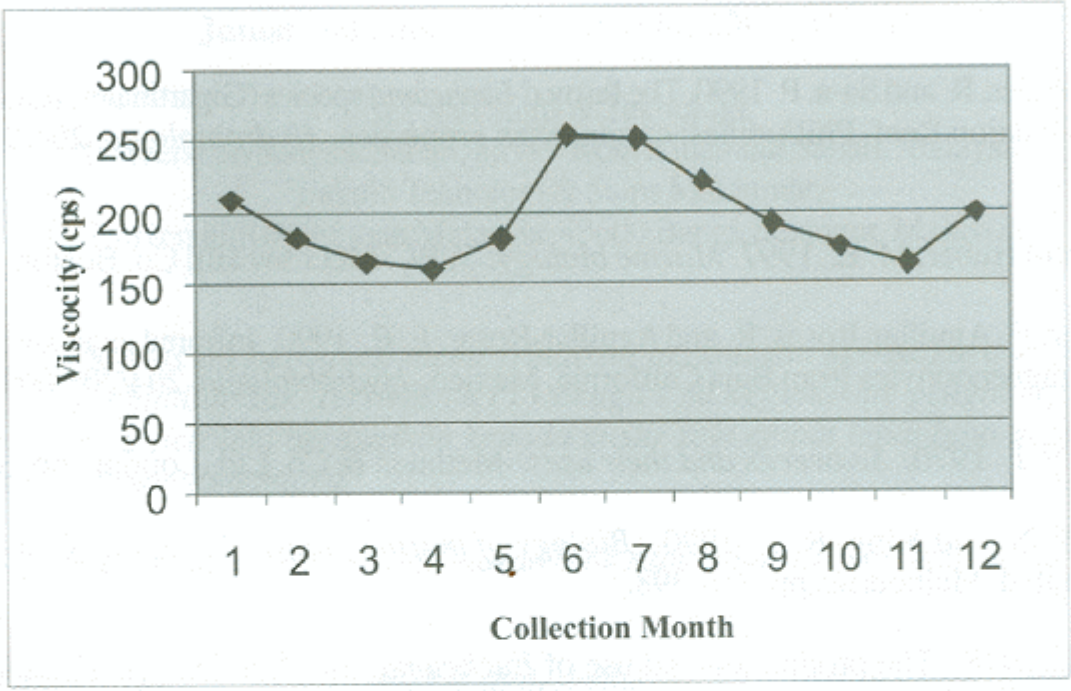


Figure 4. Annual viscosity profile of *iota*-carrageenan extracted from *E. spinosum* cultured at Teluk Lung, Pulau Balambangan, Kudat.

In conclusion, data obtained from this study indicated evidence of seasonal changes in yield and quality of carrageenan produced by *E. spinosum* cultured in Teluk Lung, Pulau Balambangan. Best harvest of *Eucheuma* for carrageenan extraction can be obtained in November/ December and June/July. Besides, its yield and quality are also consistent through-out the year as compared to the same variety cultured in the Phillipines. Information obtained from this study has shed significant light in our endeavor to better understand and improve seaweed culture at Teluk Lung, Pulau Balambangan. Hence, it is imperative that such information be taken into consideration in seaweed culture activities in Sabah.

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